Evaluation of dietary acid-binding capacity and butyrate-based feed ingredients on nursery pig performance and intestinal health parameters

by

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AN ABSTRACT OF A DISSERTATION

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Department of Agriculture
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Abstract

The 5 chapters of this dissertation involve 1) determining the effects of low acid-binding capacity (ABC) specialty soy protein sources on nursery pig performance in a commercial environment, 2) evaluating dietary ABC level on nursery pig performance and fecal dry matter (DM), 3) determining the effects of dietary acidifiers and other low ABC formulation strategies on nursery pig growth performance and fecal DM, 4) determining the effects of butyrate-based feed ingredient supplementation on nursery pig performance, fecal DM, serum chemistry, and intestinal short chain fatty acid concentration, and 5) a review to feeding wheat and wheat co-products to swine. Chapter 1 consisted of 2 experiments which used 3,317 weanling pigs to evaluate low ABC soy protein sources. In Exp. 1, low ABC soy proteins were used to replace animal protein sources. In Exp. 2, two specialty soy protein sources were used to formulate low and high acid-binding capacity at a pH of 4 (ABC-4) diets with or without pharmacological levels of Zn from ZnO. Results suggest that low ABC soy protein sources can replace animal protein sources and be used to achieve low ABC-4 diets to improve nursery pig performance and provide a similar response to pharmacological levels of Zn. Chapter 2 consisted of 2 experiments which used 660 weanling pigs to evaluate the optimal ABC-4 level of the diet. In Exp. 1, pigs were fed increasing ABC-4 levels in diets not containing pharmacological levels of Zn from ZnO plus a high ABC-4 diet with ZnO. In Exp. 2, pigs were fed increasing ABC-4 levels with two intermediate ABC-4 levels to determine if the strategy to change ABC-4 of the diet affects the response. Results suggest that the optimal ABC-4 level for nursery pig diets is 200 meq/kg from d 0 to 10 post-weaning and 250 meq/kg from d 10 to 24 post-weaning. Chapter 3 consisted of 2 experiments which used 1,025 weanling pigs to evaluate dietary acidifiers and other low ABC-4 formulation strategies on pig performance. In Exp. 1, pigs were fed four low ABC-4 diets that

were formulated using four different dietary acidifiers and two high ABC-4 diets with or without added ZnO. In Exp. 2, pigs were fed three low ABC-4 diets that were formulated using an acidifier with an additional low ABC-4 formulation strategy plus two high ABC-4 diets with or without added ZnO. Results suggest that the use of different dietary acidifiers and formulation strategies to target a low ABC-4 level can affect the response in nursery pigs, but overall, low ABC-4 diet formulation improves pig performance compared to high ABC-4 diets without ZnO. Chapter 4 consisted of 2 experiments which used 2,538 weaned pigs to evaluate butyrate-based feed ingredients. In Exp. 1, pigs were fed a positive control containing feed-grade medications and pharmacological levels of Zn and Cu, a negative control diet without pharmacological Zn and Cu, and four different butyrate-based feed additives. In Exp. 2, pigs were fed a control diet, 4 different mono-tributyrin blends, and a commercial feed additive. The results suggest that mono-tributyrin blends can improve feed efficiency, fecal DM, cecal butyrate concentration, and hematological components in nursery pigs. Chapter 5 consisted of a literature review on feeding wheat and wheat co-products to swine. Overall, wheat can offer opportunities in swine diets because of its high crude protein, amino acid, and phosphorus content relative to other cereal grains. Additionally, wheat co-products vary in their nutrient concentration due to the amount of bran, germ, and endosperm that is added back to the co-product which can ultimately affect its application in swine diets.

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Abstract

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were formulated using four different dietary acidifiers and two high ABC-4 diets with or without added ZnO. In Exp. 2, pigs were fed three low ABC-4 diets that were formulated using an acidifier with an additional low ABC-4 formulation strategy plus two high ABC-4 diets with or without added ZnO. Results suggest that the use of different dietary acidifiers and formulation strategies to target a low ABC-4 level can affect the response in nursery pigs, but overall, low ABC-4 diet formulation improves pig performance compared to high ABC-4 diets without ZnO. Chapter 4 consisted of 2 experiments which used 2,538 weaned pigs to evaluate butyrate-based feed ingredients. In Exp. 1, pigs were fed a positive control containing feed-grade medications and pharmacological levels of Zn and Cu, a negative control diet without pharmacological Zn and Cu, and four different butyrate-based feed additives. In Exp. 2, pigs were fed a control diet, 4 different mono-tributyrin blends, and a commercial feed additive. The results suggest that mono-tributyrin blends can improve feed efficiency, fecal DM, cecal butyrate concentration, and hematological components in nursery pigs. Chapter 5 consisted of a literature review on feeding wheat and wheat co-products to swine. Overall, wheat can offer opportunities in swine diets because of its high crude protein, amino acid, and phosphorus content relative to other cereal grains. Additionally, wheat co-products vary in their nutrient concentration due to the amount of bran, germ, and endosperm that is added back to the co-product which can ultimately affect its application in swine diets.

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Chapter 1 - Effects of low acid-binding capacity specialty soy protein sources on nursery pig performance in a commercial environment

Abstract

Two experiments were conducted to determine the effects of low acid-binding capacity (ABC) specialty soy protein sources on weanling pig performance. In Exp. 1, 2,260 pigs, initially 6.7 kg, were used to determine the effects of low ABC soy proteins as a replacement to poultry meal (PM) or spray-dried blood plasma (SDBP). Treatments were arranged in a 2 × 2 factorial plus a control diet fed in 2 phases. There were 20 or 21 pigs per pen with 22 replications (pens) per treatment. The control diet contained PM (AV-E Digest, XFE Products, Des Moines, IA) and SDBP (Appetein, APC Inc., Ankeny IA). Specialty soy protein concentrate (SSPC; AX3 Digest, Protekta, Newport Beach, CA) and microbial enhanced soybean meal (MESBM; MEPRO; Prairie Aquatech, Brookings, SD) were used to replace PM or PM and SDBP on a standardized ileal digestible Lys basis. From d 0 to 21 and d 0 to 42, pigs fed either soy protein source replacing PM had greater ($P \leq 0.016$) ADG and ADFI than pigs fed PM. From d 0 to 21, pigs fed SSPC had increased ($P < 0.001$) G:F compared to pigs fed MESBM and those fed either soy protein source replacing SDBP had increased ($P = 0.044$) G:F compared to pigs fed SDBP. In Exp. 2, 1,057 pigs, initially 6.2 kg, were used to determine the effects of diet ABC at a pH of 4 (ABC-4) with specialty soy proteins with or without pharmacological levels of Zn from ZnO. Experimental diets were fed in 2 phases with 22 pigs per pen and 12 replications per treatment. Dietary treatments were arranged in a 2 × 2 factorial with main effects of ABC-4 (low or high) and pharmacological levels of Zn from ZnO (105 or 2,000 mg/kg). The low ABC-4 diet without

ZnO was formulated to 150 and 200 meq/kg using SSPC in phase 1 and 2, respectively. The high ABC-4 diet used enzymatically treated soybean meal (HP 300, Hamlet Protein, Findlay, OH) which increased the ABC-4 by 127 and 104 meq/kg in phase 1 and 2, respectively. From d 0 to 21 and d 0 to 42, there was an ABC-4 × ZnO interaction ($P \leq 0.026$) observed where pigs fed low ABC-4 diets had greater ($P < 0.05$) ADG and G:F than pigs fed high ABC-4 diets without ZnO, but when diets contained added ZnO, there were no differences based on ABC-4. In conclusion, low ABC specialty soy proteins can be used to achieve low dietary ABC-4 levels to improve performance of weanling pigs and provide a similar response to those fed pharmacological levels of Zn.

Key Words: Acid-binding capacity, poultry meal, specialty soy protein, spray-dried blood plasma, nursery pig, zinc

Introduction

At the time of weaning, pigs have an underdeveloped gastrointestinal tract and their stomach hydrochloric acid secretion is limited until approximately 7 to 8 weeks of age (Pluske, 2016). This can lead to an elevated stomach pH that will negatively affect protein digestion even with the presence of high-quality protein sources (Yen, 2001). Certain ingredients common in early nursery diets possess high acid-binding capacity characteristics that can raise stomach pH and exacerbate this situation (Batonon-Alavo et al., 2016). Incorporating low acid-binding ingredients has been suggested as a method to maintain an acidic gastric environment to improve growth and health status of newly weaned pigs (Lawlor et al., 2005). However, few studies have evaluated the effect low acid-binding ingredients have on weanling pig performance and health status.

Soybean meal is the predominant protein source used in swine diets, but it contains antinutritional factors that cause a hypersensitive immune response in the gastrointestinal tract of newly weaned pigs (Li et al., 1990). This can result in increased occurrence of post-weaning diarrhea and reduced growth rate. As a result, the addition of soybean meal to the diet is limited immediately after weaning. High quality specialty protein sources, such as animal proteins or vegetable proteins with minimal anti-nutritional factors are used as partial replacements for soybean meal to meet the pig's amino acid requirements. Spray-dried blood plasma has been observed to promote growth, immune status, and gut health of newly weaned pigs (Balan et al., 2021; Kim et al., 2022). Poultry meal is a lower cost animal protein source and has been observed to have similar performance as high-quality protein sources such as spray-dried blood plasma (Zier et al., 2004; Frame et al., 2021). However, these animal protein sources have been observed to have a high acid-binding capacity at a pH of 4 (ABC-4) compared to certain vegetable protein sources (Stas et al., 2022). Vegetable proteins such as further processed soy proteins can provide an excellent amino acid profile with reduced anti-nutritional factors (Deng et al., 2023; Jang and Kim, 2023). Further processed soy proteins are associated with lower levels of glycinin and β-conglycinin that cause villus atrophy and crypt hyperplasia in the small intestine of young pigs (Kim et al., 2010). Specialty soy proteins have been observed to vary in their ABC-4 value which could have a large impact on weanling pig stomach pH (Stas et al., 2022). Incorporating low ABC-4 soy proteins may offer an added benefit not previously realized and, thus, could serve as a replacement for poultry meal and spray-dried blood plasma.

In addition to specialty protein sources, pharmacological levels of Zn from ZnO are commonly used to decrease the prevalence of post-weaning diarrhea and improve performance of weanling pigs (Bonetti et al., 2021). However, the use of ZnO at pharmacological levels has

raised concerns about antimicrobial resistance and heavy metal accumulation in the environment (Ciesinski et al., 2018; Ma et al., 2021). Therefore, other dietary strategies are being explored to maintain performance and fecal integrity in low Zn diets. Low acid-binding capacity specialty protein sources may be used to formulate to low diet ABC-4 levels and maintain performance and fecal consistency similar to pharmacological levels of Zn from ZnO.

Our hypothesis was that low ABC-4 specialty soy protein sources can serve as alternatives for more expensive, high ABC-4 animal proteins and using these soy protein sources in a low ABC-4 diet formulation strategy might be an alternative to pharmacological concentrations of Zn from ZnO.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Both experiments were conducted at commercial research sites owned and operated by New Fashion Pork, Jackson, MN. Experiment 1 was conducted in two commercial research sites. The first nursery site consisted of a single nursery room, whereas the second nursery site used two identical rooms. Experiment 2 was conducted in a single room of a commercial research site. All nursery facilities are completely enclosed, environmentally controlled, and mechanically ventilated to maintain a room temperature between 18 to 32°C for weight ranges of 5 to 30 kg.

ABC-4 analysis. For Exp. 1, a sample of poultry meal (AV-E Digest, XFE Products, Des Moines, IA), spray-dried blood plasma (Appetein, APC Inc., Ankeny, IA), specialty soy protein concentrate (AX3 Digest; Protekta; Newport Beach, CA), and microbially enhanced soybean meal (MEPRO; Prairie Aquatech; Brookings, SD) was analyzed for ABC-4. For Exp. 2, a sample

of each ingredient used in diet formulation and the complete diet was analyzed for ABC-4. All samples were analyzed at the Kansas State University Swine Nutrition Laboratory in Manhattan, KS (Table 1). Acid binding capacity analysis used the procedure outlined by Stas et al. (2022). Each diet and ingredient sample were analyzed in triplicate and the average was used for the overall ABC-4 value. In addition, water samples were collected from the nipple waterer of 3 randomly selected pens for the pH measurement of the drinking water.

Chemical analysis. Diet samples for both experiments were collected from each feeder using a feed probe to obtain a representative sample for each diet and phase. Diet samples were stored at -20°C until they were homogenized, subsampled, and submitted for analysis. Experimental protein sources were formulated according to manufacturer supplied nutrient specifications and standardized ileal digestibility (SID) coefficients of CP and AA (Table 2). A sample of specialty soy protein concentrate, microbially enhanced soybean meal, and enzymatically treated soybean meal were also collected for analysis to confirm dietary formulation. All ingredient and diet samples were submitted to the Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO) for analysis of crude protein (CP; method 990.03; AOAC International, 2006), ash, dry matter (DM; method 935.29; AOAC International, 2006), ether extract (EE; ANKOM Technology, 2004), and crude fiber (ANKOM Technology, 2005).

Experiment 1

Animals and treatment structure. The objective of Exp. 1 was to evaluate two low ABC-4 specialty soy protein sources as a replacement to poultry meal or spray-dried blood plasma in weanling pig diets. A total of 2,260 pigs (PIC TR4 × [Fast LW × PIC L02], initially 6.7 kg) were used in a 42-d trial across two facilities. In the first facility, there were 20 pigs per pen and 10

replications per treatment spread across two rooms. In the second facility, there were 21 pigs per pen and 12 replications per treatment. In total, there were 22 replications per treatment. Pigs were weaned at approximately 21-d of age and pens were randomly assigned to 1 of 5 dietary treatments based on initial body weight.

Five dietary treatments were arranged in a 2 × 2 factorial plus a control diet fed in two phases (Tables 3 and 4). The control diet contained 9.5% poultry meal in phase 1 and 2 and 4.13 (phase 1) or 2.75% (phase 2) spray-dried blood plasma. The spray dried blood plasma utilized was of bovine origin. The other 4 diets included specialty soy protein concentrate or microbially enhanced soybean meal replacing poultry meal or poultry meal and spray-dried blood plasma in the control diet. Protein sources were added to the diet to maintain a similar level of soybean meal and balanced for SID amino acids by using feed-grade amino acids. Thus, soy protein sources were added to the diet at 6 and 13% (phase 1) or 6 and 10.75% (phase 2) to replace poultry meal or poultry meal and spray-dried blood plasma, respectively. Dietary treatment structure allowed the opportunity to compare each specialty soy protein source to each other, the soy protein sources as a replacement of poultry meal, and the soy protein sources as a replacement of spray-dried blood plasma.

Experiment 2

Animal and treatment structure. The objective of Exp. 2 was to determine the effects of varying the ABC-4 diet levels by using different specialty soy protein sources with or without pharmacological levels of Zn on nursery pig performance. A total of 1,057 pigs (PIC 800 × [Fast LW × PIC L02], initially 6.2 kg) were used in a 42-d nursery trial. Pigs were weaned at approximately 21-d of age and pens of pigs were randomly assigned to 1 of 4 dietary treatments based on initial body weight. There were 22 pigs per pen and 12 replications per treatment.

Four dietary treatments were arranged in a 2 × 2 factorial with main effects of low or high ABC-4 and pharmacological levels of Zn from ZnO (105 or 2,000 mg/kg) fed in two phases (Tables 5 and 6). The low ABC-4 diets were formulated with 13.0 and 10.75% specialty soy protein concentrate in phase 1 and 2, respectively. The high ABC-4 diets were formulated with 15.85 and 13.15% enzymatically treated soybean meal (HP 300; Hamlet Protein; Findlay, OH) in phase 1 and 2, respectively, replacing the specialty soy protein concentrate on an equal SID Lys basis. Specialty soy protein concentrate and enzymatically treated soybean meal were measured to have an ABC-4 value of -13 and 753 meq/kg, respectively. The low ABC-4 diets without pharmacological levels of Zn were formulated to contain 150 and 200 meq/kg in phase 1 and 2, respectively. Replacing specialty soy protein concentrate with enzymatically treated soybean meal increased the ABC-4 of the diet by approximately 104 to 127 meq/kg. The addition of ZnO increased the ABC-4 of the diet by approximately 60 to 65 meq/kg. Diets without ZnO contained 105 mg/kg of Zn that was added individually because the vitamin trace mineral premix did not contain any Zn.

For both experiments, pigs were fed experimental diets according to a feed budget of 1.8 kg/pig and 5.4 kg/pig for phase 1 and 2, respectively. The approximate feeding duration of each experimental phase was 7-d for phase 1 (d 0 to 7) and 14-d for phase 2 (d 7 to 21). Following phase 2 diets, all pigs were fed a common corn-soybean meal-based diet for the duration of the study. Experimental diets were formulated to contain 1.36 (phase 1) and 1.42% (phase 2) SID Lys and met or exceeded other AA relative to Lys requirement estimates established by the NRC (2012). Pens of pigs were weighed and feed disappearance was measured weekly from d 7 to 42 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F). Overall growth performance was also calculated on a per pig placed basis by factoring in

potential mortality differences between treatments. Overall growth performance on a per pig placed basis was achieved by calculating BW, ADG, ADFI, and G:F based on the initial inventory at the start of each experiment. Under the circumstance where a pig died or needed to be removed from the study due to sickness or injury, the weight of the pig, the pen they originated from, the reason for removal, and date of removal was recorded for analysis. For Exp 2., on approximately d 15 of the trial, the research site experienced a porcine reproductive and respiratory syndrome virus (PRRSV) challenge. The PRRSV outbreak was confirmed by the University of Minnesota Veterinary Diagnostic Laboratory (St. Paul, MN) using real-time polymerase chain reaction of fixed lung tissue.

Statistical analysis

In both experiments, data were analyzed as a randomized complete block design with body weight serving as a blocking factor and pen as the experimental unit. Block was included in the model as a random effect. All data were analyzed using the RStudio environment [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria]. For Exp. 1, interactive and main effects of specialty soy protein source, replacement of poultry meal, and replacement of spray-dried blood plasma were tested using the lmer function. For Exp. 2, main effects of ABC-4 and added ZnO as well as their interactions were also tested using the lmer function. Removals and mortality for both experiments were analyzed as a binomial using the glmer function. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Experiment 1

There were no interactions observed between specialty soy protein source and specialty protein replacement strategy for any growth performance criteria or removals and mortality for the duration of the experiment (Table 7).

When comparing soy protein sources, in phase 1 (d 0 to 7), pigs fed microbially enhanced soybean meal tended to have increased ($P = 0.063$) ADFI compared to pigs fed specialty soy protein concentrate. In phase 2 (d 7 to 21), pigs fed specialty soy protein concentrate tended to have greater ($P = 0.098$) ADG compared to pigs fed microbially enhanced soybean meal. In both phase 2 (d 7 to 21) and the experimental period (d 0 to 21), pigs fed specialty soy protein concentrate had increased ($P < 0.001$) G:F compared to pigs fed microbially enhanced soybean meal. In the common period (d 21 to 42), pigs previously fed microbially enhanced soybean meal tended to have increased ($P = 0.079$) G:F compared to pigs previously fed specialty soy protein concentrate. Overall (d 0 to 42) and overall on a per pig placed basis, there were no differences among pigs fed the two specialty soy protein sources.

For the comparison of soy protein sources replacing poultry meal, in phase 1 (d 0 to 7), pigs fed diets containing soy protein as a replacement to poultry meal had increased ($P \leq 0.016$) BW, ADG, ADFI, and G:F compared with those fed the control diet with poultry meal. In phase 2 (d 7 to 21) and experimental period (d 0 to 21), pigs fed soy protein as a replacement to poultry meal had increased ($P < 0.001$) BW, ADG, and ADFI compared to the control diet. In the common period (d 21 to 42), pigs previously fed soy protein as a replacement to poultry meal had increased ($P = 0.044$) ADFI compared to pigs previously fed poultry meal. In the common period (d 21 to 42) and overall (d 0 to 42), pigs fed the control diet with poultry meal had

increased ($P \leq 0.029$) G:F compared to pigs fed soy protein replacing poultry meal. Overall (d 0 to 42) and overall on a per pig placed basis, pigs fed either soy protein source replacing poultry meal had increased ($P \leq 0.020$) BW, ADG, and ADFI compared to the control diet.

For the comparison of soy protein sources replacing spray-dried blood plasma, in phase 1 (d 0 to 7), pigs fed diets with soy protein replacing spray-dried blood plasma tended to have increased ($P = 0.071$) BW compared with those fed diets containing spray-dried blood plasma. In both phase 1 (d 0 to 7) and experimental period (d 0 to 21), pigs fed diets with either soy protein source replacing spray-dried blood plasma tended to have increased ($P \leq 0.097$) ADG and had increased ($P < 0.044$) G:F compared to pigs fed diets with spray-dried blood plasma. For phase 2 (d 7 to 21), the common period (d 21 to 42), overall (d 0 to 42) and overall on a per pig placed basis, there were no differences in growth performance between pigs fed diets containing spray-dried blood plasma and pigs fed diets with either soy protein source as a replacement to spray-dried blood plasma.

Experiment 2

ABC-4 analysis. All analyzed ABC-4 diet values were slightly less than calculated values. However, analyzed ABC-4 still increased with the inclusion of enzymatically treated soybean meal and ZnO. Analyzed values were on average 8.5 and 37 meq/kg lower than calculated values in diets without ZnO in phase 1 and 2, respectively. Diets with ZnO had analyzed values on average 50 and 68 meq/kg lower than calculated values for phase 1 and 2, respectively. Stas et al. (2022) also observed lower analyzed ABC-4 diet values compared to calculated ABC-4 diet values from individual ingredients analysis. The authors also indicated the addition of ZnO contributed to the discrepancy. The average pen water pH was 7.37, and because it was near neutral pH it would not be expected to influence the results of the study.

For growth performance in phase 1 (d 0 to 7), there was an ABC-4 × ZnO interaction observed ($P = 0.036$) for ADG and G:F. In diets with ZnO, pigs fed the high ABC-4 diet had greater ($P < 0.05$) ADG and a tendency for increased ($P < 0.10$) G:F compared to pigs fed the low ABC-4 diet, but when ZnO was absent, there were no differences between low and high ABC-4 diets (Table 8). In phase 2 (d 7 to 21), there was a tendency for an ABC-4 × ZnO interaction ($P \leq 0.076$) for ADG and G:F. When ZnO was not present, pigs fed the low ABC-4 diet had increased ($P < 0.05$) ADG and G:F compared to pigs fed the high ABC-4 diet, but when ZnO was added, there were no differences observed between pigs fed the low and high ABC-4 diets. In the experimental period (d 0 to 21), there was an ABC-4 × ZnO interaction observed ($P \leq 0.026$) for ADG and G:F. When ZnO was not present, pigs fed the low ABC-4 diet had increased ($P < 0.05$) ADG and G:F compared to pigs fed the high ABC-4 diet, but when ZnO was added, there were no differences between pigs fed low and high ABC-4 diets. In addition, a tendency for an ABC-4 × ZnO interaction was observed ($P = 0.091$) for ADFI. When ZnO was not present, pigs fed a low ABC-4 diet tended to have increased ($P < 0.10$) ADFI compared to pigs fed a high ABC-4 diet, but when ZnO was added, there were no differences among pigs fed low and high ABC-4 diets. There were no ABC-4 × ZnO interactions observed for the common period (d 21 to 42) or overall (d 0 to 42). However, there was an ABC-4 × ZnO interaction observed ($P \leq 0.040$) for BW and ADG overall (d 0 to 42) when data was calculated on a per pig placed basis. When ZnO was not present, pigs fed a low ABC-4 diet had greater ($P < 0.05$) ADG and tended to have increased ($P < 0.10$) BW compared to pigs fed a high ABC-4 diet, but when ZnO was added there were no differences between pigs fed low and high ABC-4 diets.

For ZnO main effects, in phase 1 (d 0 to 7) pigs fed diets containing ZnO had increased ($P \leq 0.012$) BW and ADFI compared to pigs fed diets without ZnO. In phase 2, pigs fed diets

containing ZnO tended to have increased ($P = 0.062$) ADFI compared to pigs fed diets without ZnO. In the common period (d 21 to 42), pigs previously fed diets without ZnO had increased ($P = 0.036$) G:F compared to pigs fed diets with added ZnO.

In the experimental period (d 0 to 21), there was an ABC-4 × ZnO interaction ($P = 0.025$) for total removals and mortalities and a tendency for an interaction ($P = 0.064$) for mortalities. When ZnO was not present, pigs fed a low ABC-4 diet had decreased ($P < 0.05$) mortalities and total removals and mortalities compared to pigs fed a high ABC-4 diet, but when ZnO was added there were no differences between pigs fed low and high ABC-4 diets. In the common period (d 21 to 42), there was an ABC-4 × ZnO interaction observed ($P = 0.029$) for total removals and mortalities. In diets with ZnO, pigs previously fed a high ABC-4 diet had a tendency for decreased ($P < 0.10$) total removals and mortalities compared to a low ABC-4 diet, but when ZnO was absent there were no differences between low and high ABC-4 diets. Overall (d 0 to 42), there was an ABC-4 × ZnO interaction observed ($P \leq 0.043$) for mortalities, and total removals and mortalities. When ZnO was not present, pigs fed a low ABC-4 diet had decreased ($P < 0.05$) total removals and mortalities and tended to have decreased ($P < 0.10$) mortalities compared to pigs fed a high ABC-4 diet, but when ZnO was added there were no differences between low and high ABC-4 diets.

Discussion

Further processed soy proteins are widely used in the swine industry because of their decreased allergenic components relative to conventional soybean meal and their cost competitiveness compared with animal protein sources (Long et al., 2021). However, there is limited data suggesting which soy protein source will optimize performance and health status compared to others. For example, Ma et al. (2019) observed weanling pigs fed enzymatically

treated soybean meal had improved performance compared to soy protein concentrate, whereas the opposite response was observed in a study conducted by Yang et al. (2007). Additionally, Yuan et al. (2017) observed no differences in growth comparing two different specialty soy protein sources. Specialty soy protein concentrate and microbially enhanced soybean meal may provide an added benefit compared to other specialty soy protein sources because of their low ABC-4 characteristics. However, there is limited research available evaluating this comparison. Microbially enhanced soybean meal has been observed to have comparable performance to fish meal in weanling pig diets (Sinn et al., 2017). Further evaluation of these low ABC-4 soy protein sources is warranted to understand if their low ABC-4 characteristics provide an additional opportunity in weanling pig diets.

Acid-binding capacity at a pH of 4 is the amount of hydrochloric acid required to achieve a stable pH of 4 for 1 kg of ingredient. Stas et al. (2022) observed common protein sources used for weanling pigs range widely in their ABC-4 values from -13 to 1,380 meq/kg. Among those protein sources, specialty soy protein concentrate and microbially enhanced soybean meal had the lowest ABC-4 among protein sources. In the current study, specialty soy protein concentrate and microbially enhanced soybean meal were observed to have an ABC-4 of -13 and 107 meq/kg, respectively. Whereas spray-dried blood plasma and poultry meal were analyzed to have an ABC-4 of 713 and 1,007 meq/kg, respectively. Although animal protein sources are generally considered higher quality than vegetable proteins, specialty soy protein concentrate and microbially enhanced soybean meal provide an advantage in having a lower ABC-4.

In Exp. 1, specialty soy protein concentrate and microbially enhanced soybean meal were evaluated as a replacement to poultry meal or spray-dried blood plasma in early nursery diets. Rojas et al. (2013) observed pigs fed diets containing fermented soybean meal had comparable

performance to diets containing poultry meal. In the current study, pigs fed soy proteins as a replacement to poultry meal exhibited increased gain and feed intake. The difference in growth rate observed in this study was driven by a reduction in feed intake of the diets containing poultry meal. Therefore, the soy protein sources used in this study likely had improved palatability compared to poultry meal. Previous research has suggested that the response to poultry meal is variable depending on the source and quality which can affect its palatability (Keegan et al., 2004; Babatunde et al., 2021). Spray-dried blood plasma is considered a highly palatable and digestible protein source for wenaling pigs that typically yields a more consistent response than poultry meal (Pérez-Bosque et al., 2016). Deng et al. (2022) observed a linear reduction in body weight, gain, feed intake, and feed efficiency when replacing poultry meal, fish meal, and spray-dried bovine blood plasma with a soy protein concentrate. Similarly, Espinosa et al. (2022) observed decreased ADG and G:F when soy protein concentrate replaced spray-dried bovine blood plasma. The authors also observed looser stool associated with the pigs fed soy protein concentrate compared to spray-dried bovine blood plasma. However, the soy proteins used by Deng et al. (2022) and Espinosa et al. (2022) were different sources than the low acid-binding soy proteins used in Exp. 1. In the current study, replacing spray-dried blood plasma with low ABC-4 soy proteins numerically increased ADG and significantly improved feed efficiency during the experimental period. The results of these studies indicate that a low acid-biding capacity soy protein may be used to replace high quality animal proteins without diminishing performance which previously has not been observed.

Although both specialty soy protein concentrate and microbially enhanced soybean meal have low ABC-4 values compared to other specialty protein sources, specialty soy protein concentrate is approximately 120 meq/kg lower than microbially enhanced soybean meal. This

difference in ABC-4 could explain the results of Exp. 1. Pigs fed specialty soy protein concentrate had improved feed efficiency during the experimental period compared to microbially enhanced soybean meal. It can be theorized that specialty soy protein concentrate was able to maintain a more acidic environment in the stomach of weanling pigs than microbially enhanced soybean meal which led to the improvement in nutrient utilization. Ruckman et al. (2020) also observed differences in weanling pig feed efficiency when feeding different specialty soy protein sources. While the authors did not measure ABC-4 of the specialty soy protein sources, the specialty soy protein source with the lowest ash content had the most improved feed efficiency from d 0 to 14 post-weaning. Ash content of ingredients has been observed to be positively correlated with ABC-4 values because of increased concentrations of cationic minerals such as Ca and Mg (Jasaitis et al., 1987; Lawlor et al., 2005). While low ABC-4 characteristics of soy protein sources may explain the improvements in feed efficiency, Exp. 1 diets were not formulated to a specific ABC-4 level which was our intention with formulation in Exp. 2.

In Exp. 2, specialty soy protein concentrate was used to target an ABC-4 level of 150 meq/kg from d 0 to 7 post-weaning followed by 200 meq/kg from d 7 to 21 post-weaning. These ABC-4 targets were based on European recommendations to improve performance and fecal consistency (Gilles Langeoire, personal communication, November 4, 2022). Lawlor et al. (2006) observed pigs fed an ABC-4 level of 180 meq/kg for 28 d post weaning resulted in the most improved growth performance compared to higher ABC-4 levels. In the current study, the ABC-4 level of the diet was adjusted between the two dietary phases to coincide with the developing gastrointestinal tract of weanling pigs. Additionally, phase feeding is a common practice in the swine industry to match the nutrient requirements and digestive capabilities of

weanling pigs. In contrast to Exp. 1, enzymatically treated soybean meal was used as the other soy protein source because of its high ABC-4 value. Replacing specialty soy protein concentrate with enzymatically treated soybean meal on an SID Lys basis increased the ABC-4 level of the diet by 127 and 104 meq/kg in phase 1 and 2, respectively. All diets contained 105 mg/kg of Zn provided by an organic Zn source. The use of pharmacological levels of Zn from ZnO also further increased the ABC-4 of the diet because of the high ABC-4 value for ZnO. Although a high ABC-4 is thought to be more detrimental to early nursery pigs, ZnO has multiple modes of action to overcome the increase in ABC-4. Zinc oxide reduces pro-inflammatory cytokines, increase anti-inflammatory cytokines, maintains healthy morphology of the gastrointestinal tract, prevents attachment of pathogenic bacteria, and reduces intestinal permeability (Huang et al., 1999; Stark et al., 2013; Peng et al., 2020). However, the use of pharmacological levels of Zn have been banned in the European Union and Canada. Therefore, low ABC-4 formulation may be a potential alternative to pharmacological levels of Zn.

The results of Exp. 2 indicate that utilizing specialty soy protein concentrate to achieve a low ABC-4 level can improve performance and reduce mortality compared to a high ABC-4 soy protein. The PRRS disease challenge was identified on d 15 of the experiment. The health challenge in combination with a commercial setting allowed the opportunity to observe removal and mortality differences that are typically not able to be observed in smaller studies. The use of specialty soy protein concentrate to achieve low ABC-4 diets not only improved growth rate, but also led to fewer occurrences of morbidity and mortality under the PRRSV disease challenge in diets without pharmacological levels of Zn from ZnO. The low ABC-4 diets also led to similar performance as the diets containing ZnO. Therefore, low ABC-4 formulation strategies may be used as a replacement to pharmacological levels of Zn from ZnO to maintain performance and

health status. Strategies such as low CP diets or high fiber diets have been suggested as alternatives to ZnO, but these strategies are generally associated with a reduction in performance (Yan et al., 2017; Batson et al., 2021; Bonetti et al., 2021). To our knowledge, this study is one of the first to illustrate the effect low acid-binding soy proteins have on nursery pig performance and mortality. While low ABC-4 specialty soy proteins aid in lowering the overall diet ABC-4 level, other diet aspects need be considered when formulating to specific ABC-4 targets. Other diet strategies such as lactose sources with low ABC-4 values, lower diet Ca levels, and use of acidifiers must also be incorporated to achieve an ABC-4 level similar to the current study. The current study observed a benefit to an ABC-4 level of 150 and 200 meq/kg compared to 267 and 304 meq/kg from d 0 to 7 and 7 to 21 post-weaning, respectively. However, the current study only evaluated two ABC-4 levels and more research is needed to understand what the optimal ABC-4 level is of a complete diet for a specific age or weight range on a weanling pig.

In summary, the results of Exp. 1 indicate that using low acid-binding soy protein can improve performance of nursery pigs compared to animal protein sources. The results of Exp. 2 indicate that low acid-binding soy proteins can be used to target a low ABC-4 level to improve performance and reduce removals and mortality in diets not containing pharmacological levels of Zn from ZnO. Low acid-binding capacity specialty soy protein sources offer an advantage in weanling pig diets as a method to maintain an acidic gastric environment in the stomach of the newly weaned pig to improve performance and maintain health.

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Table 1.1 Analyzed acid-binding capacity at a pH of 4 (ABC-4) of ingredients used in experimental diets[1]

Item	Initial pH	ABC-4 (meq/kg)
Ingredients, %		
Corn	6.38	84
Soybean meal (46.8% CP)	7.05	602
Cereal blend	6.08	107
Poultry meal[2]	6.74	1,007
Spray-dried blood plasma[3]	6.99	713
Specialty soy protein concentrate[4]	3.95	-13
Microbially enhanced soybean meal[5]	5.01	107
Enzymatically treated soybean meal[6]	6.29	753
Oat groats	6.44	107
Crystalline lactose	7.09	53
Fat blend	6.60	363
Beef tallow	4.90	16
Limestone	9.68	18,384
Dicalcium phosphate (19% P)	7.03	3,673
Salt	5.24	10
L-Lys	5.80	80
DL-Met	6.13	140
L-Thr	5.54	160
L-Trp	5.11	120
L-Val	5.53	193
L-Ile	5.76	200
Choline chloride	5.21	40
Zinc oxide	9.88	21,863
Organic Zn (21.0%)[7]	7.14	12,820
Vitamin-trace mineral premix	4.67	1,727
Organic Mn (20.0%)[7]	8.22	2,347

Fumaric acid	2.34	-10,862

[1] Acid-binding cpacity-4 was determined by the amount of 0.1 N HCl required to lower the initial pH of a sample to a stable pH of 4.00 ± 0.04. Each sample was analyzed in triplicate. Only experimental protein sources were analyzed for Exp.1, whereas all ingredients included in experimental diets were analyzed for Exp. 2

[2] AV-E Digest, XFE Products, Des Moines, IA.

[3] Appetein, APC Inc., Ankeny, IA

[4] AX3 Digest, Protekta, Newport Beach, CA.

[5] MEPRO, Prairie Aquatech, Brookings, SD.

[6] HP 300, Hamlet Protein, Findlay, OH.

[7] Zinpro, Eden Prairie, MN.

Table 1.2 Manufacturer provided nutrients specification of protein sources used in diet formulation (as-fed basis)[1]

Item	Poultry meal[2]	Spray-dried blood plasma[3]	Specialty soy protein concentrate[4]	Microbially enhanced soybean meal[5]	Enzymatically treated soybean meal[6]
Item, %					
DM	93.90	92.00	92.00	95.50	93.00
CP	51.35	77.85	68.00	70.60	56.00
EE	16.05	2.00	---	0.85	3.00
Crude fiber	1.30	---	4.00	5.40	4.00
Ash	13.70	8.70	3.60	1.30	7.00
Amino acids					
Arg	3.73	4.39	4.90	4.47	3.97
Cys	0.99	2.60	1.00	1.13	0.82
His	1.29	2.53	1.70	1.74	1.47
Ile	2.04	2.69	3.20	3.57	2.75
Leu	3.61	7.39	5.40	5.71	4.32
Lys	3.02	6.90	4.10	4.12	3.45
Met	0.89	0.79	1.00	1.02	0.77
Phe	2.44	4.25	3.70	3.70	2.90
Thr	2.04	4.47	2.70	2.78	2.18
Trp	0.44	1.41	0.90	1.00	0.77
Val	2.52	5.12	3.40	3.62	2.82
Analyzed values, %[7]					
DM	90.11	88.68	93.97	94.53	93.74

CP	48.38	76.21	64.94	66.59	53.53
EE	12.76	---	0.02	---	1.12
Crude fiber	1.45	---	6.02	4.75	4.71
Ash	14.58	8.04	2.94	1.35	7.11

[1] Nutrient specifications of each protein source used in diet formulation. Values were obtained from manufacturer of each protein source.

[2] AV-E Digest, XFE Products, Des Moines, IA.

[3] Appetein, APC Inc., Ankeny, IA

[4] AX3 Digest, Protekta, Newport Beach, CA.

[5] MEPRO, Prairie Aquatech, Brookings, SD.

[6] HP 300, Hamlet Protein, Findlay, OH.

[1] A sample of each soy protein source was submitted to the Agricultural Experiment Station Chemical Laboratories (University of Missouri-Columbia, Columbia, MO).

Table 1.3 Phase 1 diet composition (as-fed basis), Exp. 1[1]

		Specialty soy protein conentrate[2]		Microbially enhanced soybean meal[3]	
Item	Control	Replacing poultry meal	Replacing poultry meal and blood plasma	Replacing poultry meal	Replacing poultry meal and blood plasma
Ingredients, %					
Corn	44.10	46.34	43.36	46.17	42.96
Soybean meal (46.8% CP)	18.59	18.58	18.54	18.74	18.87
Cereal blend	13.10	13.10	13.10	13.10	13.10
Poultry meal[4]	9.50	---	---	---	---
Specialty soy protein conentrate[2]	---	6.00	13.00	---	---
Microbially enhanced soybean meal[3]	---	---	---	6.00	13.00
Spray-dried blood plasma[5]	4.13	4.13	---	4.13	---
Oat groats	3.75	3.75	3.75	3.75	3.75
Crystalline lactose	1.38	1.38	1.38	1.38	1.38
Fat blend	2.00	2.00	2.00	2.00	2.00
Beef tallow	0.50	0.50	0.50	0.50	0.50
Limestone	0.38	0.60	0.33	0.68	0.53
Dicalcium phosphate (19% P)	0.23	1.05	1.40	0.95	1.15
Salt	---	0.25	0.45	0.33	0.63
L-Lys	0.42	0.42	0.42	0.42	0.42
DL-Met	0.20	0.21	0.22	0.20	0.20
L-Thr	0.26	0.27	0.26	0.26	0.25

L-Trp	0.08	0.07	0.07	0.06	0.05
L-Val	0.11	0.10	0.06	0.10	0.05
L-Ile	0.11	0.09	---	0.08	---
Copper chloride	0.04	0.04	0.04	0.04	0.04
Choline chloride	0.03	0.03	0.03	0.03	0.03
Zinc oxide	0.30	0.30	0.30	0.30	0.30
Vitamin E	0.05	0.05	0.05	0.05	0.05
Vitamin-trace mineral premix[6]	0.25	0.25	0.25	0.25	0.25
Benzoic acid	0.50	0.50	0.50	0.50	0.50
Organic Mn (20.0%)[7]	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100

Table 1.3 (cont.)

Item	Control	Specialty soy protein concentrate[2]		Microbially enhanced soybean meal[3]	
		Replacing poultry meal	Replacing poultry meal and blood plasma	Replacing poultry meal	Replacing poultry meal and blood plasma
SID amino acids, %					
Lys	1.36	1.36	1.36	1.36	1.36
Ile:Lys	59	59	61	60	63
Leu:Lys	105	108	113	109	114
Met:Lys	33	33	36	33	35
Met and Cys:Lys	56	56	56	56	56
Thr:Lys	66	66	66	66	66
Trp:Lys	22.2	22.2	22.3	22.1	22.2
Val:Lys	70	70	70	70	70
His:Lys	34	35	36	35	36
Total Lys, %	1.55	1.52	1.51	1.52	1.52
NE NRC, kcal/kg	2,330	2,410	2,381	2,401	2,361
SID Lys:NE, g/Mcal	5.84	5.65	5.71	5.67	5.76
CP, %	22.0	21.3	22.6	21.5	23.0
Ca, %	0.59	0.59	0.59	0.59	0.59
P, %	0.55	0.58	0.62	0.55	0.59
STTD P, %	0.43	0.43	0.43	0.43	0.43

Analyzed values, %					
DM	89.09	89.34	89.12	89.57	90.00
CP	19.98	21.99	22.18	22.27	22.34
Crude fat	4.14	3.70	3.49	3.17	3.13
Crude fiber	2.10	2.06	2.49	2.27	2.45
Ash	4.71	4.61	4.62	4.62	4.86

[1] Phase 1 diets were fed according to a feed budget of 1.8 kg/pig.

[2] AX3 Digest; Protekta; Newport Beach, CA.

[3] MEPRO; Prairie Aquatech; Brookings, SD.

[4] AV-E Digest; XFE Products; Des Moines, IA.

[5] Appetein; APC Inc., Ankeny, IA.

[6] Provided per kg of diet: 10,472 IU vitamin A; 83 IU vitamin E; 4 mg vitamin K; 0.03 mg vitamin B12; 40 mg niacin; 30 mg pantothenic acid; 8 mg riboflavin; 100 mg iron; 16 mg copper; 0.5 mg iodine; 0.30 mg selenium. Vitamin-trace mineral premix contained Optiphos PLUS (Huvepharma Inc.; Peachtree City, Georgia) which provided an estimated release of 0.12% STTD P with 647 FTU/kg.

[7] Zinpro, Eden Prairie, MN.

Table 1.4 Phase 2 diet composition (as-fed basis), Exp. 1[1]

		Specialty soy protein concentrate[2]		Microbially enhanced soybean meal[3]	
Item	Control	Replacing poultry meal	Replacing poultry meal and blood plasma	Replacing poultry meal	Replacing poultry meal and blood plasma
Ingredients, %					
Corn	52.72	54.96	53.03	54.77	52.71
Soybean meal (46.8% CP)	22.76	22.76	22.60	22.91	22.87
Cereal blend	5.00	5.00	5.00	5.00	5.00
Poultry meal[4]	9.50	---	---	---	---
Specialty soy protein concentrate[2]	---	6.00	10.75	---	---
Microbially enhanced soybean meal[3]	---	---	---	6.00	10.75
Spray-dried blood plasma[5]	2.75	2.75	---	2.75	---
Crystalline lactose	1.38	1.38	1.38	1.38	1.38
Fat blend	2.00	2.00	2.00	2.00	2.00
Beef tallow	0.50	0.50	0.50	0.50	0.50
Limestone	0.53	0.75	0.55	0.83	0.70
Dicalcium phosphate (19% P)	0.23	1.05	1.30	0.95	1.10
Salt	0.18	0.43	0.58	0.50	0.70
L-Lys	0.49	0.49	0.49	0.49	0.49
DL-Met	0.24	0.25	0.24	0.25	0.24
L-Thr	0.27	0.27	0.26	0.27	0.25
L-Trp	0.09	0.08	0.08	0.07	0.07

L-Val	0.16	0.16	0.13	0.16	0.13
L-Ile	0.10	0.07	---	0.07	---
Copper chloride	0.04	0.04	0.04	0.04	0.04
Choline chloride	0.03	0.03	0.03	0.03	0.03
Zinc oxide	0.28	0.28	0.28	0.28	0.28
Vitamin E	0.01	0.01	0.01	0.01	0.01
Vitamin-trace mineral premix[6]	0.25	0.25	0.25	0.25	0.25
Benzoic acid	0.50	0.50	0.50	0.50	0.50
Organic Mn (20.0%)[7]	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100

Table 1.4 (cont.)

Item	Control	Specialty soy protein concentrate[2]		Microbially enhanced soybean meal[3]	
		Replacing poultry meal	Replacing poultry meal and blood plasma	Replacing poultry meal	Replacing poultry meal and blood plasma
SID amino acids, %					
Lys	1.42	1.42	1.42	1.42	1.42
Ile:Lys	58	57	58	59	60
Leu:Lys	103	106	109	107	110
Met:Lys	36	35	36	35	36
Met and Cys:Lys	56	56	56	56	56
Thr:Lys	66	66	66	66	66
Trp:Lys	22.3	22.3	22.3	22.2	22.4
Val:Lys	71	71	71	71	71
His:Lys	33	34	34	34	34
Total Lys, %	1.61	1.58	1.57	1.58	1.58
NE NRC, kcal/kg	2,414	2,493	2,474	2,485	2,458
SID Lys:NE, g/Mcal	5.88	5.70	5.74	5.72	5.77
CP, %	22.6	22.0	22.8	22.2	23.1
Ca, %	0.64	0.64	0.64	0.64	0.64
P, %	0.56	0.58	0.61	0.55	0.56

STTD P, %	0.43	0.43	0.43	0.43	0.43
Analyzed values, %					
DM	88.47	88.03	88.23	88.48	88.65
CP	21.33	22.87	22.44	22.40	22.95
Crude fat	4.13	3.51	2.88	3.21	2.93
Crude fiber	1.87	2.29	2.38	2.13	2.22
Ash	4.90	4.61	4.91	4.85	5.02

[1] Phase 2 diets were fed according to a feed budget of 5.4 kg/pig.

[2] AX3 Digest; Protekta; Newport Beach, CA.

[3] MEPRO; Prairie Aquatech; Brookings, SD.

[4] AV-E Digest; XFE Products; Des Moines, IA.

[5] Appetein; APC Inc., Ankeny, IA.

[6] Provided per kg of diet: 10,472 IU vitamin A; 83 IU vitamin E; 4 mg vitamin K; 0.03 mg vitamin B12; 40 mg niacin; 30 mg pantothenic acid; 8 mg riboflavin; 100 mg iron; 16 mg copper; 0.5 mg iodine; 0.30 mg selenium. Vitamin-trace mineral premix contained Optiphos PLUS (Huvepharma Inc.; Peachtree City, Georgia) which provided an estimated release of 0.12% STTD P with 647 FTU/kg.

[7] Zinpro, Eden Prairie, MN.

Table 1.5 Phase 1 diet composition (as-fed basis), Exp. 2[1]

	No ZnO		Added ZnO[2]	
Item	Low ABC-4[3]	High ABC-4[3]	Low ABC-4[3]	High ABC-4[3]
Ingredients, %				
Corn	42.81	39.85	42.51	39.55
Soybean meal (46.8% CP)	18.58	18.58	18.58	18.58
Cereal blend	13.10	13.10	13.10	13.10
Specialty soy protein concentrate[4]	13.00	---	13.00	---
Enzymatically treated soybean meal[5]	---	15.85	---	15.85
Oat groats	3.75	3.75	3.75	3.75
Crystalline lactose	1.38	1.38	1.38	1.38
Fumaric acid	1.27	1.27	1.27	1.27
Fat blend	2.00	2.00	2.00	2.00
Beef tallow	0.50	0.50	0.50	0.50
Limestone	0.32	0.40	0.32	0.40
Dicalcium phosphate (19% P)	1.40	1.20	1.40	1.20
Salt	0.45	0.63	0.45	0.63
L-Lys	0.42	0.42	0.42	0.42
DL-Met	0.22	0.24	0.22	0.24
L-Thr	0.26	0.28	0.26	0.28
L-Trp	0.07	0.06	0.07	0.06
L-Val	0.06	0.08	0.06	0.08
Copper chloride	0.04	0.04	0.04	0.04
Choline chloride	0.03	0.03	0.03	0.03
Zinc oxide	---	---	0.30	0.30
Organic Zn (21.0%)[6]	0.05	0.05	0.05	0.05
Vitamin E	0.05	0.05	0.05	0.05
Vitamin-trace mineral premix[7]	0.25	0.25	0.25	0.25
Organic Mn (20.0%)[6]	0.02	0.02	0.02	0.02
Total	100	100	100	100

Table 1.5 (cont.)

Item	No ZnO		Added ZnO[2]	
	Low ABC-4[3]	High ABC-4[3]	Low ABC-4[3]	High ABC-4[3]
SID amino acids, %				
Lys	1.36	1.36	1.36	1.36
Ile:Lys	61	60	61	60
Leu:Lys	113	108	113	108
Met:Lys	36	37	36	37
Met and Cys:Lys	56	56	56	56
Thr:Lys	66	66	66	66
Trp:Lys	22.3	22.2	22.3	22.2
Val:Lys	70	70	70	70
His:Lys	36	36	36	36
Total Lys, %	1.51	1.52	1.51	1.52
NE, kcal/kg	2,368	2,350	3,359	2,341
SID Lys:NE, g/Mcal	5.75	5.79	5.76	5.81
CP, %	22.5	22.4	22.5	22.4
Ca, %	0.58	0.59	0.58	0.59
P, %	0.61	0.62	0.61	0.62
STTD P, %	0.46	0.46	0.46	0.46
ABC-4, meq/kg	150	277	215	342
Analyzed values, %				
DM	88.84	88.78	89.02	89.01
CP	22.01	22.38	22.29	23.23
Crude fat	3.74	4.06	3.87	3.73
Crude fiber	2.52	2.52	2.45	2.28
Ash	4.89	5.35	4.94	5.52
ABC-4, meq/kg	143	267	167	290

[1] Phase 1 diets were fed according to a feed budget of 1.8 kg/pig.

[2] Diets contained 2,265 mg/kg Zn from ZnO.

[3] Acid-binding capacity at a pH of 4. The amount of hydrochloric acid required to achieve a stable pH of 4 for 1 kg of diet.

[4] AX3 Digest; Protekta; Newport Beach, CA.

[5] HP 300; Hamlet Protein; Findlay, OH

[6] Zinpro, Eden Prairie, MN.

[7] Provided per kg of diet: 10,472 IU vitamin A; 83 IU vitamin E; 4 mg vitamin K; 0.03 mg vitamin B12; 40 mg niacin; 30 mg pantothenic acid; 8 mg riboflavin; 100 mg iron; 16 mg copper; 0.5 mg iodine; 0.30 mg selenium. Vitamin-trace mineral premix contained Optiphos PLUS (Huvepharma Inc.; Peachtree City, Georgia) which provided an estimated release of 0.12% STTD P with 647 FTU/kg.

Table 1.6 Phase 2 diet composition (as-fed basis), Exp. 2[1]

	No ZnO		Added ZnO[2]	
Item	Low ABC-4[3]	High ABC-4[3]	Low ABC-4[3]	High ABC-4[3]
Ingredients, %				
Corn	52.32	49.87	52.04	49.59
Soybean meal (46.8% CP)	22.65	22.65	22.65	22.65
Cereal blend	5.00	5.00	5.00	5.00
Specialty soy protein concentrate[4]	10.75	---	10.75	---
Enzymatically treated soybean meal[5]	---	13.15	---	13.15
Crystalline lactose	1.38	1.38	1.38	1.38
Fumaric acid	1.38	1.38	1.38	1.38
Fat blend	2.00	2.00	2.00	2.00
Beef tallow	0.50	0.50	0.50	0.50
Limestone	0.56	0.63	0.56	0.63
Dicalcium phosphate (19% P)	1.30	1.13	1.30	1.13
Salt	0.58	0.70	0.58	0.70
L-Lys	0.49	0.49	0.49	0.49
DL-Met	0.25	0.26	0.25	0.26
L-Thr	0.26	0.27	0.26	0.27
L-Trp	0.08	0.08	0.08	0.08
L-Val	0.13	0.14	0.13	0.14
Copper chloride	0.04	0.04	0.04	0.04
Choline chloride	0.03	0.03	0.03	0.03
Zinc oxide	---	---	0.28	0.28
Organic Zn (21.0%)[6]	0.05	0.05	0.05	0.05
Vitamin E	0.01	0.01	0.01	0.01
Vitamin-trace mineral premix[7]	0.25	0.25	0.25	0.25
Organic Mn (20.0%)[6]	0.02	0.02	0.02	0.02
Total	100	100	100	100

Table 1.6 (cont.)

Item	No ZnO		Added ZnO[2]	
	Low ABC-4[3]	High ABC-4[3]	Low ABC-4[3]	High ABC-4[3]
SID amino acids, %				
Lys	1.42	1.42	1.42	1.42
Ile:Lys	58	57	58	57
Leu:Lys	109	106	109	105
Met:Lys	37	37	37	37
Met and Cys:Lys	56	56	56	56
Thr:Lys	63	63	63	63
Trp:Lys	22.3	22.2	22.3	22.2
Val:Lys	71	71	71	71
His:Lys	34	34	34	34
Total Lys, %	1.57	1.58	1.57	1.58
NE, kcal/kg	2,456	2,443	2,449	2,434
SID Lys:NE, g/Mcal	5.78	5.82	5.80	5.84
CP, %	22.7	22.6	22.7	22.6
Ca, %	0.64	0.64	0.64	0.64
P, %	0.61	0.61	0.61	0.61
STTD P, %	0.46	0.46	0.46	0.46
ABC-4, meq/kg	200	304	261	365
Analyzed values, %				
DM	89.05	89.02	89.20	89.91
CP	22.03	22.66	22.35	22.30
Crude fat	3.88	4.08	3.94	3.90
Crude fiber	2.16	2.00	2.14	2.18
Ash	4.86	5.45	5.49	5.55
ABC-4, meq/kg	170	260	197	293

[1] Phase 2 diets were fed according to a feed budget of 5.4 kg/pig.

[2] Diets contained 2,121 mg/kg Zn from ZnO.

[3] Acid-binding capacity at a pH of 4. The amount of hydrochloric acid required to achieve a stable pH of 4 for 1 kg of diet.

[4] AX3 Digest; Protekta; Newport Beach, CA.

[5] HP 300; Hamlet Protein; Findlay, OH.

[6] Zinpro, Eden Prairie, MN.

[7] Provided per kg of diet: 10,472 IU vitamin A; 83 IU vitamin E; 4 mg vitamin K; 0.03 mg vitamin B12; 40 mg niacin; 30 mg pantothenic acid; 8 mg riboflavin; 100 mg iron; 16 mg copper; 0.5 mg iodine; 0.30 mg selenium. Vitamin-trace mineral premix contained Optiphos PLUS (Huvepharma Inc.; Peachtree City, Georgia) which provided an estimated release of 0.12% STTD P with 647 FTU/kg.

Table 1.7 Effects of specialty soy protein products to replace poultry meal and blood plasma in nursery diets, Exp. 1[1]

		Specialty soy protein conetrate[2]		Microbially enhanced soybean meal[3]			P^4 =		
Item	Control	Replacing poultry meal	Replacing poultry meal and blood plasma	Replacing poultry meal	Replacing poultry meal and blood plasma	SEM	Soy protein source[5]	Replacing poultry meal with soy source[6]	Replacing blood plasma with soy source[7]
BW, kg									
d 0	6.7	6.7	6.7	6.7	6.7	0.06	0.954	0.796	0.819
d 7	6.9	7.0	7.1	7.0	7.1	0.06	0.503	0.001	0.071
d 21	10.9	11.6	11.7	11.4	11.6	0.13	0.210	<0.001	0.130
d 42	20.5	21.1	21.4	21.1	21.2	0.31	0.548	0.028	0.392
d 42 per pig placed[8]	19.4	20.0	20.5	20.3	20.6	0.38	0.555	0.020	0.135
Day 0 to 7									
ADG, g	21	41	49	44	53	5.0	0.477	<0.001	0.075
ADFI, g	65	88	81	89	98	4.6	0.063	<0.001	0.789
G:F g/kg	304	463	594	444	548	51.4	0.515	0.016	0.020
Day 7 to 21									
ADG, g	286	323	326	309	320	6.1	0.098	<0.001	0.251
ADFI, g	351	389	387	382	396	6.3	0.846	<0.001	0.332
G:F, g/kg	814	831	843	810	808	8.2	<0.001	0.495	0.529
Experimental period (d 0 to 21)									
ADG, g	197	228	233	220	230	4.6	0.229	<0.001	0.097

ADFI, g	254	288	284	283	296	4.9	0.439	<0.001	0.341
G:F, g/kg	772	794	819	775	778	8.0	<0.001	0.162	0.044
Day 21 to 42									
ADG, g	451	443	457	458	456	12.3	0.370	0.964	0.428
ADFI, g	666	691	706	692	702	14.0	0.838	0.044	0.236
G:F, g/kg	676	640	646	661	649	8.9	0.079	0.002	0.735
Overall (d 0 to 42)									
ADG, g	322	334	343	337	342	6.9	0.890	0.016	0.135
ADFI, g	456	486	492	484	497	8.1	0.834	0.001	0.181
G:F, g/kg	704	686	698	695	688	6.6	0.874	0.029	0.630
Overall (d 0 to 42) per pig placed									
ADG, g[9]	303	318	329	323	331	8.7	0.552	0.019	0.139
ADFI, g[10]	442	474	481	473	487	9.1	0.710	<0.001	0.162
G:F, g/kg	682	669	683	679	677	9.1	0.725	0.389	0.409
Removals, %[11]	4.5	4.2	2.7	2.7	2.0	0.94	0.245	0.376	0.231
Mortality, %[12]	1.1	0.9	1.3	1.3	1.1	0.58	0.851	0.988	0.851
Removals and mortality, %	5.6	5.1	4.0	4.0	3.1	1.08	0.416	0.349	0.333

[1] A total of 2,260 pigs (initially 6.7 ± 0.06 kg) across two facilities were used in a 42-d nursery trial. A total of 5 dietary treatments were used with two different specialty soy protein sources replacing poultry meal or poultry meal and spray-dried blood plasma. In the first facility, there were 20 pigs per pen and 10 pens per treatment spread across two barns. In the second facility, there were 21 pigs per pen and 12 replications per treatment. In total, there were 22 replications per treatment.

[2] AX3 Digest; Protekta; Newport Beach, CA.

[3] MEPRO; Prairie Aquatech; Brookings, SD.

[4] There were no soy protein source × poultry meal and spray-dried blood plasma replacement interaction observed ($P > 0.05$) throughout the duration of the study.

[5] Compares the mean of pigs fed diets containing specialty soy protein concentrate to those fed diets containing microbially enhanced soybean meal.

[6] Compares the control to the mean of each poultry meal replacement diet.

[7] Compares the mean of pigs fed diets with soy protein sources replacing poultry meal to the mean of pigs fed diets with soy protein sources replacing poultry meal and spray-dried blood plasma.

[8] BW per pig placed = final pen ending weight ÷ pigs placed

[9] ADG per pig placed = (final pen ending weight – initial pen weight) ÷ (Pigs initially placed × days of trial)

[10] ADFI per pig placed = (total feed intake) ÷ (Pigs initially placed × days of trial).

[11] Percentage of pigs removed from original pen.

[12] Percentage of pigs that died or were euthanized in original pen.

Table 1.8 Interactive effects of acid-binding capacity-4 (ABC-4) and pharmacological ZnO on nursery pig performance, Exp. 2[1]

	No ZnO		Added ZnO[2]			*P* =		
Item:	Low ABC-4[3]	High ABC-4[3]	Low ABC-4[3]	High ABC-4[3]	SEM	ABC-4 × ZnO	ABC-4	ZnO
BW, kg								
d 0	6.2	6.2	6.2	6.2	0.11	0.196	0.775	0.324
d 7	6.3	6.3	6.5	6.6	0.11	0.303	0.228	<0.001
d 21	9.2	8.9	9.5	9.7	0.20	0.230	0.642	0.008
d 42	17.5	17.8	17.8	17.8	0.29	0.617	0.747	0.623
d 42 per pig placed[4]	14.4	12.8	14.3	15.1	0.60	0.040	0.466	0.050
Day 0 to 7								
ADG, g[5]	23	14	39	59	6.8	0.036	0.415	<0.001
ADFI, g	72	71	80	85	4.2	0.461	0.671	0.012
G:F, g/kg	318	163	459	702	92.9	0.036	0.634	0.001
Day 7 to 21								
ADG, g[6]	191	145	193	196	13.1	0.063	0.106	0.043
ADFI, g	268	233	271	276	12.1	0.102	0.223	0.062
G:F, g/kg[6]	702	607	709	708	26.3	0.076	0.070	0.045
Experimental period (d 0 to 21)								
ADG, g[6]	133	98	140	149	9.5	0.026	0.175	0.004
ADFI, g	200	176	205	210	8.7	0.091	0.247	0.027
G:F, g/kg[6]	655	543	676	707	29.3	0.017	0.166	0.003

Day 21 to 42								
ADG, g	374	394	372	370	10.9	0.306	0.409	0.218
ADFI, g	521	542	528	531	11.1	0.397	0.299	0.830
G:F, g/kg	716	727	703	697	10.0	0.408	0.827	0.036
Overall (d 0 to 42)								
ADG, g	246	229	247	254	8.5	0.152	0.563	0.124
ADFI, g	351	338	354	363	8.7	0.202	0.788	0.106
G:F, g/kg	699	673	696	701	12.1	0.192	0.367	0.308
Overall (d 0 to 42) per pig placed								
ADG, g[6,7]	201	158	193	213	13.4	0.024	0.394	0.080
ADFI, g[8]	308	291	327	320	12.4	0.640	0.307	0.047
G:F, g/kg	643	583	587	672	0.063	0.240	0.835	0.789
Experimental period (d 0 to 21)[9]								
Removals, %[10]	3.3	6.2	3.4	3.0	1.55	0.239	0.417	0.285
Mortality, %[6,11]	5.5	11.2	7.3	6.6	2.23	0.064	0.167	0.557
Removals and mortality, %[6]	9.0	17.7	11.0	9.8	2.49	0.025	0.102	0.241
Day 21 to 42								
Removals, %[10]	2.3	4.5	2.6	0.0	1.47	0.999	0.999	0.999
Mortality, %[11]	5.9	7.5	7.2	5.5	1.87	0.301	0.950	0.822
Removals and mortality, %	8.1	12.0	9.8	5.4	2.37	0.029	0.690	0.175
Overall (d 0 to 42)								
Removals, %[10]	5.3	9.9	5.7	3.0	1.92	0.021	0.986	0.037

Mortality, %[11]	11.1	17.8	14.0	11.7	2.46	0.043	0.354	0.554
Removals and mortality, %[6]	16.5	27.7	19.7	14.8	2.88	0.002	0.327	0.076

[1] A total of 1,057 pigs (initial BW of 6.2 ± 0.11 kg) were used in a 42-d nursery trial. A total of 4 dietary treatments were arranged in a 2 × 2 factorial design consisting of low or high ABC-4 of the diet and absence or presence of ZnO. There were 22 pigs per pen and 12 replications per treatment.

[2] Diets contained 2,265 and 2,121 mg/kg of Zn from ZnO in phase 1 and 2, respectively.

[3] The low ABC-4 diets were formulated with a specialty soy protein concentrate (AX3 Digest; Protekta; Newport Beach, CA) and the high ABC-4 diets were formulated with enzymatically treated soybean meal (HP 300; Hamlet Protein; Findlay, OH). The low ABC-4 diet without ZnO was formulated to a value of 150 and 200 meq/kg in phase 1 and 2, respectively. The addition of ZnO increased the ABC-4 of the diet by approximately 60 to 65 meq/kg. Replacing specialty soy protein concentrate with enzymatically treated soybean meal increased the ABC-4 of the diet by 104 to 127 meq/kg.

[4] BW per pig placed = final pen ending weight ÷ pigs placed

[5] ABC-4 effect without ZnO, $P > 0.10$. ABC-4 effect with ZnO, $P < 0.05$.

[6] ABC-4 effect without ZnO, $P < 0.05$. ABC-4 effect with ZnO, $P > 0.10$.

[7] ADG per pig placed = (final pen ending weight – initial pen weight) ÷ (Pigs initially placed × days of trial)

[8] ADFI per pig placed = (total feed intake) ÷ (Pigs initially placed × days of trial).

[9] The research site experienced a PRRS disease challenge on approximately d 15 of the trial.

[10] Percentage of pigs that were removed into a hospital pen.

[11] Percentage of pigs that died or euthanized in original pen or hospital pen after being removed.

Chapter 2 - Evaluation of dietary acid-binding capacity on nursery pig growth performance and fecal dry matter

Abstract

Two experiments were conducted to evaluate dietary acid-binding capacity-4 (ABC-4) level on weanling pig performance and fecal dry matter (DM). In both experiments, there were 5 pigs per pen and 12 replications per dietary treatment fed in two phases. In Exp. 1, 360 barrows, initially 5.9 kg, were allotted to 1 of 6 treatments. Five treatments were formulated with increasing ABC-4 ranging from 150 (low ABC-4) to 312 meq/kg (high ABC-4) in phase 1 and 200 (low ABC-4) to 343 meq/kg (high ABC-4) in phase 2. The low ABC-4 diet was formulated with specialty soy protein concentrate (SSPC; AX3 Digest, Protekta, Newport Beach, CA) and a combination of dietary acidifiers. Increasing ABC-4 was achieved by progressively replacing SSPC with enzymatically treated soybean meal (ESBM; HP 300, Hamlet Protein, Findlay, OH) on a standardized ileal digestible (SID) Lys basis and decreasing acidifiers. Diets were formulated to contain 100 mg/kg of Zn. For the sixth treatment, pharmacological levels of Zn from ZnO was added to the high ABC-4 diet. From d 0 to 23 and d 0 to 38, increasing ABC-4 increased then decreased (quadratic, $P \leq 0.046$) ADG and ADFI. On d 10 and 17, increasing ABC-4 decreased (linear, $P \leq 0.022$) fecal DM. From d 0 to 23, pigs fed the diet containing ZnO had increased ($P \leq 0.009$) BW, ADG and ADFI compared to the high ABC-4 diet without ZnO. In Exp 2, 300 pigs, initially 6.0 kg, were allotted to 1 of 5 dietary treatments. Diet 1 was a low ABC-4 diet formulated to 200 and 250 meq/kg in phase 1 and 2, respectively. Two intermediate ABC-4 levels were formulated by either replacing SSPC with ESBM on an SID Lys basis (diet 2) or removing acidifiers (diet 3). Diet 4 was a high ABC-4 diet formulated by replacing SSPC and removing acidifiers to increase ABC-4. Diet 5 was diet 4 but with pharmacological levels of

Zn from ZnO. Diets 1 through 4 contained 110 mg/kg of Zn. From d 0 to 24 and d 0 to 38, increasing ABC-4 decreased (linear, $P \leq 0.046$) G:F. On d 10 and 24, increasing ABC-4 decreased (linear, $P \leq 0.005$) fecal DM. From d 0 to 24, pigs fed the diet containing ZnO had increased ($P \leq 0.047$) BW, ADG, ADFI, and G:F compared to the high ABC-4 diet without ZnO. In conclusion, an ABC-4 of 200 meq/kg from d 0 to 10 post-weaning and 250 meq/kg from d 10 to 24 post-weaning improves pig growth performance and fecal DM for compared to higher ABC-4 diets. Low ABC-4 diet formulation can improve performance and fecal DM of weanling pigs in diets not containing ZnO.

Key Words: Acid-binding capacity, acidifiers, fecal dry matter, specialty soy protein, weanling pig

Introduction

At weaning, pigs experience a dramatic change from a liquid milk-based diet in the form of sow's milk to a dry cereal grain-based diet. At this age, weanling pigs have limited capacity to produce enough hydrochloric acid to maintain an optimal stomach pH which can persist until 7 to 8 weeks of age (Manners, 1976; Xu and Cranwell, 1990; Pluske, 2016). Certain ingredients used in weanling pig diets can further raise stomach pH because of their high acid-binding capacity characteristics (Lawlor et al., 2005; Batonon-Alavo et al., 2016; Wang et al., 2023). Elevated stomach pH is associated with decreased nutrient utilization and accumulation of undigested protein in the small and large intestine (Yen, 2001). This provides an opportunity for pathogenic organisms to compromise the digestive tract leading to increased post-weaning diarrhea presence and enteric disorders (Jha and Berrocoso, 2016).

Low acid-binding capacity diets have been suggested to aid in reducing the occurrence of post-weaning diarrhea and subsequently improving performance (Lawlor et al., 2005). The concept of acid-binding capacity involves incorporating low acid-binding ingredients and avoiding high acid-binding ingredients to maintain an acidic gastric environment in young pigs' stomach. Acid-binding capacity at a pH of 4 (ABC-4) is the amount of hydrochloric acid required to achieve a stable pH of 4 for 1 kg of ingredient or diet (Lawlor et al., 2005; Stas et al., 2022; Wang et al., 2023). Careful selection of low ABC-4 ingredients in nursery diets can assist newly weaned pigs in maintaining an acidic gastric environment. Recent research has shown improvements in weanling pig performance and fecal dry matter (DM) with low ABC-4 formulation strategies such as lowering the calcium carbonate levels of the diet (Warner et al., 2023), using low ABC-4 specialty protein sources (Stas et al., 2024), and incorporating dietary acidifiers (Lee et al., 2021a). However, the ideal ABC-4 level of the diet for a specific age or weight range of newly weaned pigs is unclear.

Historically, pharmacological levels of Zn from ZnO have been used to improve performance and reduce the severity of post-weaning diarrhea of weanling pigs. Pharmacological levels of Zn prevent attachment of pathogenic bacteria in the intestine, increase anti-inflammatory cytokines, increase integrity of tight junctions, and decrease pro-inflammatory cytokines (Starke et al., 2013; Peng et al., 2020). However, there are increasing regulations on the use of pharmacological levels of Zn because of antimicrobial resistance and environmental concerns (Ciesinski et al., 2018). As a result, other dietary formulation strategies are being evaluated to help maintain performance and reduce the incidence of diarrhea with low Zn diets.

Therefore, the objective of these studies was to determine the ideal ABC-4 level of the diet for weanling pigs and determine if the method used to achieve a low ABC-4 level affects

this response. Our hypothesis was that low ABC-4 levels will improve growth performance and fecal DM and diets with low ABC-4 values might be an alternative for pharmacological levels of added Zn.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. The experiments were conducted at the Kansas State University Segregated Early Weaning facility (Exp. 1) and Kansas State University Swine Teaching and Research Center (Exp. 2) located in Manhattan, KS. Within each facility, experiments were conducted in two identical nursery rooms which were completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. Pens (1.2 × 1.2 m) had tri-bar floors and allowed approximately 0.30 m^2/pig.

ABC-4 analysis. A sample of each ingredient and complete diet were analyzed for ABC-4 at the Kansas State University Swine Nutrition Laboratory in Manhattan, KS (Table 1). Analysis was accomplished by the procedure outlined by Stas et al. (2022). Each ingredient and diet were analyzed in triplicate and the average of each analysis was used for the overall analyzed ABC-4 value. Individual ingredient ABC-4 values were collected prior to each experiment and were utilized in diet formulation. Complete diets were analyzed to confirm dietary ABC-4 formulation from ingredient values.

Procedure

In both experiments, pigs were weaned at approximately 21-d of age and placed in pens of 5 pigs each based on initial body weight (BW). Pigs were fed phase 1 experimental diets from

placement until d 10 and then phase 2 experimental diets from d 10 to 24. Following phase 2, all pigs were fed a common phase 3 diet until the completion of each study. In Exp. 1, all pigs were transitioned to a common diet on d 23. In both experiments, diets were formulated to contain 1.36 (phase 1) and 1.35% (phase 2), standardized ileal digestible (SID) Lys and met or exceeded other AA relative to Lys requirement estimates established by the National Research Council (NRC, 2012). Salt inclusion was also adjusted in all experimental diets to achieve the same sodium level of 0.40 and 0.35% in phases 1 and 2, respectively. All diets were manufactured at the Kansas State University O.H. Kruse Feed and Technology Center in Manhattan, KS. Phase 1 diets were fed in pellet form, and phase 2 and 3 diets were fed in meal form.

Individual pig weights and feed disappearance were measured on d 10, 17, 23 (d 24 in Exp. 2), 31, and 38 to determine ADG, ADFI, and G:F. Fecal samples were collected on d 10, 17 and 23 (d 24 for Exp. 2) to determine percentage fecal DM from the same three medium weight pigs per pen. After collection, fecal samples were dried at 55°C in a forced air oven for 48 h and the ratio of dried to wet fecal weight determined the percentage fecal DM.

Experiment 1

Animals and treatment structure. The objective of Exp. 1 was to determine the influence of increasing ABC-4 level on weanling pig performance and fecal DM. A total of 360 barrows (DNA 200 × 400; initially 5.9 ± 0.16 kg BW) were used in a 38-d trial between two identical nursery rooms. At weaning, pens of pigs were randomly allotted to 1 of 6 dietary treatments with 12 replications per treatment. Dietary treatments consisted of five diets with increasing levels of ABC-4 and a sixth high ABC-4 diet that contained pharmacological levels of Zn from ZnO fed in two phases (Tables 2 and 3). The lowest ABC-4 diet was formulated to 150 and 200 meq/kg in phase 1 and 2, respectively. To achieve the low ABC-4 diet, specialty soy protein concentrate

(AX3 Digest; Protekta; Newport Beach, CA) with an individual ingredient ABC-4 of -13 meq/kg was added at 12.5 and 10.0% of the diet in phase 1 and 2, respectively. Fumaric acid (Primary Products Ingredients Americas LLC, Decatur, IL) and formic acid (Amasil NA, BASF; Florham Park, NJ) were also included at 0.50 and 0.48% of the diet, respectively for both phase 1 and phase 2 diets. Fumaric acid and formic acid had individual ABC-4 values of -10,873 and -8,287 meq/kg, respectively. Increasing ABC-4 was achieved by progressively replacing specialty soy protein concentrate with enzymatically treated soybean meal (753 meq/kg; HP 300; Hamlet Protein; Findlay, OH) on an SID Lys basis and decreasing the levels of fumaric acid and formic acid proportionally for each diet. The first five treatment diets with increasing ABC-4 levels contained 110 mg/kg of Zn provided by the trace mineral premix and did not contain pharmacological levels of Zn. The sixth high ABC-4 diet contained 3,000 and 2,000 mg/kg of Zn from ZnO in phase 1 and 2, respectively. The addition of ZnO increased the ABC-4 of the diet by 87 and 55 meq/kg for phase 1 and 2, respectively. Dietary ABC-4 value increased by approximately 50 meq/kg as diets increased from the lowest to the highest ABC-4 levels for each diet between phases 1 and 2.

Experiment 2

Animal and treatment structure. The objective of Exp. 2 was to determine the effects of increasing the ABC-4 level of the diet and the strategy to do so (replacing specialty soy protein sources or decreasing dietary acidifiers) on weanling pig performance and fecal DM. A total of 300 pigs (DNA 241 × 600; initially 6.0 ± 0.02 kg BW) were used in a 38-d trial between two identical nursery rooms. At weaning, pens of pigs were randomly allotted to 1 of 5 dietary treatments with 12 replications per treatment. Dietary treatments consisted of a single low ABC-4 diet, two intermediate ABC-4 diets, and two high ABC-4 diets fed in two phases (Tables 4 and

5). For Diet 1, to achieve the low ABC-4 diet, specialty soy protein concentrate (AX3 Digest; Protekta; Newport Beach, CA) was included at 9.38 and 7.50% of the diet in phase 1 and 2, respectively. Fumaric acid and formic acid were also included at 0.38 and 0.36% of the diet, respectively, for both phase 1 and phase 2 diets. Ultimately, diet 1 was formulated to 200 and 250 meq/kg in phase 1 and 2, respectively. Diets 2 and 3, the intermediate ABC-4 diets, were formulated by utilizing two different strategies to determine if ingredient selection to change ABC-4 affects the response. Diet 2 was achieved by replacing specialty soy protein concentrate with enzymatically treated soybean meal (797 meq/kg; HP 300; Hamlet Protein; Findlay, OH) on an SID Lys-basis and resulted in dietary ABC-4 values of 290 and 322 meq/kg for phase 1 and 2, respectively. Diet 3 was achieved by removing the organic acids and resulted in dietary ABC-4 values of 271 and 321 meq/kg for phase 1 and 2, respectively. Diet 4, the high ABC-4 diet, was formulated by replacing specialty soy protein concentrate with enzymatically treated soybean meal on an SID Lys-basis and removing the organic acids resulting in dietary ABC-4 values of 362 and 394 meq/kg for phase 1 and 2, respectively. Diets 1 through 4 contained 110 mg/kg of Zn provided by the trace mineral premix. Diet 5 was formulated the same as diet 4 but with the addition of 3,000 and 2,000 mg/kg of Zn from ZnO in phase 1 and 2, respectively. The addition of ZnO increased dietary ABC-4 values by 87 and 54 meq/kg for phase 1 and 2, respectively.

Statistical analysis

For both experiments, data were analyzed as a completely randomized design using the RStudio environment (Version 1.3.1093, RStudio, Inc., Boston, MA) and R programming language [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria] with pen as the experimental unit. Nursery room in each trial was included as a random effect. In both experiments, linear and quadratic effects of diet ABC-4 were tested using

the lmer function. The contrast coefficients were developed using the assumption that the treatments were evenly spaced based on dietary ABC-4 values. The effect of ZnO was tested by a pairwise comparison between the high ABC-4 diets with or without added ZnO. A multiple comparison Dunnett's test was performed with pigs fed the high ABC-4 diets with ZnO serving as the control compared all other treatments. Fecal DM were analyzed using the fixed effects of day, treatment, and the associated interaction accounting for repeated measures over time. In Exp. 2, the effect of strategy to increase diet ABC-4 was tested by a pairwise comparison of both intermediate ABC-4 diets. Differences between treatments and day (where appropriate) as well as their interaction were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

ABC-4 analysis

All analyzed ABC-4 dietary values were less than calculated values regardless of treatment, phase, or experiment. For Exp. 1, analyzed ABC-4 values for diets without ZnO were on average 31 and 57 meq/kg lower than calculated values for phases 1 and 2, respectively. The high ABC-4 diet with ZnO was 59 and 72 meq/kg lower for analyzed ABC-4 compared to calculated ABC-4. For Exp. 2, analyzed ABC-4 values for diets without ZnO were on average 25 meq/kg lower than calculated values for both phases 1 and 2. The high ABC-4 diet with ZnO was 62 and 51 meq/kg lower for analyzed ABC-4 compared to calculated ABC-4. Although lower than calculated values, all analyzed ABC-4 values still increased incrementally as designed in both experiments.

Experiment 1

Increasing ABC-4 value in the diet had no effect on growth performance during phase 1 (d 0 to 10; Table 6). In phase 2 (d 10 to 23) and the experimental period (d 0 to 23), BW, ADG and ADFI increased and then decreased (quadratic, $P \leq 0.045$) with increasing ABC-4 levels with the greatest BW, ADG and ADFI for pigs fed the second (203 and 247 meq/kg in phases 1 and 2 respectively) and third (256 and 296 meq/kg in phases 1 and 2, respectively) ABC-4 levels. In addition, pigs fed increasing ABC-4 had decreased (linear, $P \leq 0.002$) G:F. In the common period (d 23 to 38) and overall (d 0 to 38), a similar quadratic response was observed ($P \leq 0.046$) with the highest BW, ADG, ADFI (overall only), and G:F being observed at the second (203 and 247 meq/kg in phases 1 and 2, respectively), and third (256 and 296 meq/kg in phases 1 and 2, respectively) ABC-4 levels. On d 10 and 17, increasing ABC-4 decreased (linear, $P \leq 0.022$) fecal DM.

For pigs fed the high ABC-4 diet containing ZnO, in phase 1 (d 0 to 10), phase 2 (d 10 to 23), and the experimental period (d 0 to 23), pigs had increased ($P \leq 0.034$) BW, ADG, and ADFI (phase 1 and the experimental period only) compared to pigs fed the high ABC-4 diet without ZnO. In the experimental period (d 0 to 23), pigs fed the high ABC-4 diet containing ZnO also tended to have increased ($P = 0.060$) G:F compared to pigs fed the high ABC-4 diet without ZnO. In the common period (d 23 to 38) and overall (d 0 to 38), there were no differences among pigs fed high ABC-4 diets with or without added ZnO. In addition, there were no differences between pigs fed high ABC-4 diets with or without added ZnO for fecal DM on d 10, 17, and 23.

Using the Dunnett's test multiple comparison procedure, in phase 1 (d 0 to 10), pigs fed the diet containing ZnO had increased ($P \leq 0.045$) BW and ADG compared to pigs fed the first

(150 and 200 meq/kg in phases 1 and 2, respectively), fourth (311 and 343 meq/kg in phases 1 and 2, respectively), and fifth (365 and 390 meq/kg in phases 1 and 2, respectively) ABC-4 levels. Pigs fed the diet containing ZnO had increased ($P = 0.001$) ADFI compared to the first (150 and 200 meq/kg in phases 1 and 2, respectively) ABC-4 level. In phase 2 (d 10 to 23), there were no multiple comparison differences observed. In the experimental period (d 0 to 23), pigs fed the diet containing ZnO had increased ($P \leq 0.035$) BW compared to the fourth (311 and 343 meq/kg in phases 1 and 2, respectively) and fifth (365 and 390 meq/kg in phases 1 and 2, respectively) ABC-4 levels. Pigs fed the diet containing ZnO had increased ($P \leq 0.043$) ADG compared to the first (150 and 200 meq/kg in phases 1 and 2, respectively), fourth (311 and 343 meq/kg in phases 1 and 2, respectively), and fifth (365 and 390 meq/kg in phases 1 and 2, respectively) ABC-4 levels. Pigs fed the diet containing ZnO had increased ($P \leq 0.040$) ADFI compared to the first (150 and 200 meq/kg in phases 1 and 2, respectively) and fifth (365 and 390 meq/kg in phases 1 and 2, respectively) ABC-4 levels. In the common period (d 23 to 38) and overall (d 0 to 38), there were no multiple comparison differences observed. On d 17, pigs fed the second (203 and 247 meq/kg in phases 1 and 2, respectively) and third (256 and 296 meq/kg in phases 1 and 2, respectively) ABC-4 level had increased ($P \leq 0.032$) fecal DM compared to the diet containing ZnO.

Experiment 2

No differences in growth performance were observed with increasing ABC-4 values for phase 1 (d 0 to 10; Table 7). For phase 2 (d 10 to 24), pigs fed increasing ABC-4 had decreased (linear, $P = 0.026$) ADG and tended to have decreased (linear, $P = 0.098$) ADFI. In the experimental period (d 0 to 24), pigs fed increasing ABC-4 levels had decreased (linear, $P = 0.024$) G:F and tended to have decreased (linear, $P \leq 0.064$) BW and ADG. In the common

period (d 24 to 38), pigs previously fed the intermediate ABC-4 diets had increased (quadratic, $P \leq 0.024$) ADFI but decreased (quadratic, $P \leq 0.021$) G:F compared to the low and high ABC-4 diets. Overall (d 0 to 38), increasing ABC-4 decreased (linear, $P = 0.046$) G:F. On d 10 and d 24, increasing ABC-4 levels decreased (linear, $P \leq 0.005$) fecal DM.

There were no differences in growth performance throughout the experiment between the intermediate ABC-4 levels where ABC-4 was altered by changing specialty soy protein source or by decreasing acidifier concentrations. On d 10, pigs fed the specialty soy protein replacement strategy had increased ($P = 0.003$) fecal DM compared to the acidifier removal strategy.

For pigs fed the high ABC-4 diet containing ZnO, in phase 1 (d 0 to 10), pigs tended to have increased ($P = 0.085$) G:F compared to the high ABC-4 diet without ZnO. In phase 2 (d 10 to 24) and the experimental period (d 0 to 24), pigs fed the high ABC-4 diet containing ZnO had increased ($P \leq 0.047$) BW, ADG, ADFI, and G:F compared to pigs fed the high ABC-4 diet without ZnO. However in the common period, pigs previously fed the high ABC-4 diet without ZnO had increased ($P \leq 0.006$) ADG and G:F compared to the high ABC-4 diet with ZnO. Overall (d 0 to 38), there were no differences in growth performance between pigs fed high ABC-4 diets with or without added ZnO. Pigs fed the high ABC-4 diet with ZnO had increased ($P < 0.001$) d 10 fecal DM and tended to have increased ($P = 0.077$) d 17 fecal DM compared to pigs fed the high ABC-4 diet without ZnO.

In phase 1 (d 0 to 10), there were no differences observed using the Dunnett's multiple comparison procedure. In phase 2 (d 10 to 24), pigs fed the diet containing ZnO had increased ($P \leq 0.049$) ADG and ADFI compared to the high ABC-4 diet without ZnO. In the experimental period (d 0 to 24), pigs fed the diet containing ZnO had increased ($P \leq 0.018$) ADG and G:F compared to the high ABC-4 diet without ZnO. In the common period (d 24 to 38), pigs

previously fed all ABC-4 levels had increased ($P \leq 0.017$) ADG compared to the diet containing ZnO. Pigs previously fed the intermediate ABC-4 level replacing the soy protein source had increased ($P = 0.023$) ADFI compared to the diet containing ZnO. Pigs previously fed the low and high ABC-4 diets had increased ($P \leq 0.018$) G:F compared to the diet containing ZnO. Overall (d 0 to 38), there were no multiple comparison differences observed. On d 10, pigs fed the diet containing ZnO had increased ($P < 0.001$) fecal DM compared to the intermediate ABC-4 level without acidifiers and the high ABC-4 level.

Discussion

In pigs, protein digestion begins in the stomach with the conversion of pepsinogen to pepsin. This conversion occurs rapidly at a pH below 4 but occurs more slowly as pH of the stomach increases (Suiryanrayna and Ramana, 2015). Undigested protein promotes microbial fermentation and the occurrence of post-weaning diarrhea followed by diminished growth, morbidity, and potentially even mortality (Rist et al., 2013). Proper acidification of the stomach is the primary defense mechanism to pathogenic organisms and the occurrence of post-weaning diarrhea (Heo et al., 2012). Therefore, formulating diets to a low ABC-4 value can maintain an acidic environment in the stomach of newly weaned pig and might provide some of the same benefits as pharmacological levels of Zn.

The concept of low ABC-4 ingredients and diets is not new and has been evaluated previously (Lawlor et al., 2005; Batonon-Alavo et al., 2016; Stas et al., 2022). However, suggested benefits to low ABC-4 ingredients and diet formulation appear to be mostly anecdotal. Previous studies have observed benefits to lowering the overall ABC-4 level of the diet (Lawlor et al., 2006; Warner et al., 2023; Stas et al., 2024). However, these studies consist of wide ranges

in diet ABC-4 and there is no data available on the exact ABC-4 level required for weanling pigs of a specific age or weight range.

Low ABC-4 diet formulation can be achieved by various strategies such as careful selection of grain source, P source, specialty protein and lactose sources, decreasing Ca concentration of the diet, increasing the use of feed-grade amino acids, selection of premix carriers, and incorporation of dietary acidifiers (Lawlor et al., 2005; Batonon-Alavo et al., 2016; Lu et al., 2016; Stas et al., 2022; Wang et al., 2023). Many of these strategies have been evaluated previously but not necessarily as strategies to target specific ABC-4 values. Bloxham et al. (2018) observed that progressively replacing corn (ABC-4 of 84 meq/kg) with wheat (ABC-4 of 43 meq/kg) in diets linearly increased G:F. Stas et al. (2024) observed pigs fed low ABC-4 specialty soy proteins had comparable ADG to pigs fed spray-dried blood plasma. The authors also observed low ABC-4 specialty soy proteins increased G:F and reduced removals and mortalities compared to high ABC-4 specialty soy proteins. Nessmith et al. (1997) observed pigs fed crystalline lactose (ABC-4 of 53 meq/kg) had increased G:F compared to pigs fed dried whey powder (ABC-4 of 440 meq/kg). Warner et al. (2023) observed decreasing the calcium carbonate (ABC-4 of 18,384 meq/kg) level in the diet linearly increased ADG and G:F. Lastly, a meta-analysis by Wang et al. (2022) reported dietary acidifiers (associated with negative ABC-4 values) consistently improved pig performance compared to those fed diets without acidifiers.

The current research chose to focus on specialty soy protein sources and dietary acidifiers to change ABC-4 values. Specialty protein sources have been observed to have a wide range in ingredient ABC-4 values (Stas et al., 2022). Dietary acidifiers are a simple method to lower the ABC-4 level without affecting other ingredients or nutrients within the diet. In Exp. 1, the low ABC-4 diet was targeted based on the results from Stas et al. (2022). While low ABC-4 specialty

protein sources and dietary acidifiers help to lower the ABC-4 level of the diet, these strategies were not enough by themselves to achieve the lowest level of 150 and 200 meq/kg in phases 1 and 2, respectively. Crystalline lactose, monocalcium phosphate, and low Ca levels were also incorporated to achieve the low ABC-4 diet. These additional strategies did not vary between treatments but do provide more examples of strategies to lower overall diet ABC-4 values.

In Exp. 1, growth performance for pigs fed diets without ZnO was most improved at 203 and 247 meq/kg in phases 1 and 2, respectively. However, fecal DM was most improved at 150 and 200 meq/kg in phases 1 and 2, respectively. Therefore, the lowest ABC-4 level may be more beneficial to decrease prevalence and severity of post-weaning diarrhea. It is possible that the 0.98% inclusion of fumaric, and formic acid in the low ABC-4 diet, or low ABC-4 itself may have affected palatability ultimately decreasing feed intake and subsequent ADG. Previous studies have also observed decreased ADFI when dietary acidifier inclusion becomes too high (Che et al., 2012; Ahmed et al., 2014). While the current study observed the most benefit at approximately 200 and 250 meq/kg in phase 1 and 2, respectively, lower ABC-4 values may provide more of a benefit if dietary formulation doesn't negatively impact palatability. For example, feeding wheat or barley-based diets would allow a lower target ABC-4 because of their lower ingredient ABC-4 value compared to corn (Lawlor et al., 2005; Stas et al., 2022). Lawlor et al. (2006) observed that pigs fed an ABC-4 value of 180 meq/kg in barley- and wheat-based diets resulted in the most improved growth performance compared to higher ABC-4 levels. This observation explains European ABC-4 recommendations of 150 meq/kg from d 0 to 10 and 200 meq/kg from d 10 to 24 post-weaning (personal communication, Gilles Langeorie, November 4, 2022). Ultimately, results of Exp. 1 indicate the ideal ABC-4 level to improve both growth performance and fecal DM is approximately 200 meq/kg from d 0 to 10 and 250 meq/kg from d

10 to 23 postweaning in corn-based diets. However, validation of this observation was warranted to determine if improvements in performance and fecal DM associated with Exp. 1 are because of the low ABC-4 level, specialty soy protein concentrate vs. enzymatically treated soybean meal, or the combination of dietary acidifiers.

In Exp. 2, the low ABC-4 diet was formulated to 200 meq/kg in phase 1 and 250 meq/kg in phase 2 and was very similar in composition to the diet with the same ABC-4 diet levels in Exp. 1. The two intermediate ABC-4 levels in Exp. 2 allowed the opportunity to determine if improvements in performance and fecal DM were because of ABC-4 level or because of an ingredient effect (specialty soy protein concentrate vs. enzymatically treated soybean meal or level of dietary acidifiers). The results of Exp. 2 confirm the observations seen in Exp. 1, where the most improved growth performance and fecal DM was observed at 200 and 250 meq/kg from d 0 to 10 and 10 to 24 postweaning, respectively. In addition, improvements in growth performance appear to be directly associated with the ABC-4 level. Strategy to change dietary ABC-4 level did not affect the response with pigs fed the two intermediate ABC-4 levels having similar growth performance. However, removing dietary acidifiers reduced d 10 fecal DM, whereas replacing the specialty soy protein source did not. This agrees with previous research where the addition of organic acids in weanling pig diets aids in improving fecal consistency (Choi et al., 2020; Lee et al., 2021b). The use of organic acids not only reduces dietary ABC-4 but has been observed to decrease populations of pathogenic bacteria such as *E. coli and Salmonella* (Grilli et al., 2015). Therefore, acidifiers may need to be present in low ABC-4 diets without pharmacological levels of ZnO to obtain improvements in fecal consistency initially after weaning. This observation was not observed once pigs were transitioned to phase 2 diets.

The use of pharmacological levels of Zn from ZnO has been established as a method to improve growth, feed intake, nutrient digestion, and lower the incidence of diarrhea (Satessa et al., 2020; Szuba-Trznadel et al., 2021). However, ZnO's use at pharmacological levels has raised several concerns such as toxicity from prolonged administration, heavy metal accumulation in the environment, modification of bacterial populations, and increased antimicrobial resistance (Bonetti et al., 2021). Therefore, much attention has been directed to alternative dietary strategies to try to elicit some of the same beneficial effects of pharmacological level of Zn for newly weaned pig.

The ideal ABC-4 value of 200 and 250 meq/kg in phase 1 and 2, respectively, also resulted in similar performance as pigs fed diets containing pharmacological levels of Zn in both experiments. A study conducted by Landero et al. (2024) reported that feeding low ABC-4 diets were able to recover 33% of lost performance from removing pharmacological levels of Zn from the diet. However, pigs fed low ABC-4 diets without ZnO were never able to reach equal performance as diets containing pharmacological levels of Zn. In contrast, pigs fed an ABC-4 level of 200 and 250 meq/kg in phase 1 and 2, respectively, had on average only a 4% reduction in ADG and similar G:F compared to pigs fed diets containing ZnO during the experimental period for both of the current studies. However, overall in both experiments, pigs fed 200 and 250 meq/kg ABC-4 diets in phase 1 and 2, respectively, had 2% greater ADG and G:F compared to the high ABC-4 diets containing ZnO. Ultimately, once pigs transitioned to the common diet, pigs previously fed pharmacological levels on Zn from ZnO experienced a reduction in growth which led to the low ABC-4 diets being numerically better overall. Previous research had also observed a reduction in growth performance once pigs transition off diets with pharmacological levels of Zn from ZnO (Batson et al., 2021).

In summary, results of both experiments indicate the optimal ABC-4 value of corn-based diets for weanling pigs is approximately 200 meq/kg from d 0 to 10 post weaning and 250 meq/kg from d 10 to 24 post-weaning. Lower ABC-4 levels are associated with further improvements in fecal DM, but also poorer performance as palatability may be negatively affected. The use of dietary acidifiers to lower the ABC-4 level has greater benefits for increasing fecal DM than replacing the specialty soy protein source. In addition, if the use of ZnO at pharmacological levels is not available, low ABC-4 diet formulation is an option to improve performance and fecal DM of newly weaned pigs.

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Table 2.1 Analyzed acid-binding capacity at a pH of 4 (ABC-4) of ingredients used in experimental diets[1]

Ingredient, %	Initial pH	ABC-4 (meq/kg)
Corn	6.50	84
Soybean meal	6.97	602
Specialty soy protein concentrate[2]	3.97	-13
Enzymatically treated soybean meal[3]	6.50	753
Crystalline lactose	5.85	53
Spray-dried bovine plasma	7.61	713
Limestone	9.61	18,384
Monocalcium phosphate	4.04	73
Salt	6.13	15
L-Lys	5.79	83
DL-Met	5.74	137
L-Thr	5.49	160
L-Trp	5.21	120
L-Val	5.37	193
Fumaric acid	2.34	-10,873
Formic acid[4]	3.02	-8,287
ZnO	9.40	21,863
Vitamin premix	5.49	10,767
Trace mineral premix	5.49	7,867

[1] Acid-binding cpacity-4 was determined by the amount of 0.1 N HCl required to lower the initial pH of a sample to a stable pH of 4.00 ± 0.04. Dietary acidifiers were titrated with the same procedure but with 0.1 N NaOH to raise the initial pH. Each sample was analyzed in triplicate.

[2] AX3 Digest, Protekta, Newport Beach, CA.

[3] HP 300, Hamlet Protein, Findlay, OH.

[4] Amasil NA, BASF, Florham Park, NJ.

Table 2.2 Phase 1 diet composition (as-fed basis), Exp. 1[1]

	Calculated ABC-4, meq/kg					
Item:	150	203	256	311	365	452[2]
Ingredients, %						
Corn	51.67	51.25	50.82	50.35	49.87	49.47
Soybean meal	11.95	11.95	11.95	11.95	11.95	11.95
Crystalline lactose	15.00	15.00	15.00	15.00	15.00	15.00
Specialty soy protein concentrate[3]	12.50	9.38	6.25	3.13	---	---
Enzymatically treated soybean meal[4]	---	3.80	7.55	11.40	15.20	15.20
Spray-dried bovine plasma	2.50	2.50	2.50	2.50	2.50	2.50
Corn oil	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.35	0.35	0.36	0.37	0.38	0.38
Monocalcium phosphate	1.00	0.95	0.90	0.88	0.83	0.83
Salt	0.65	0.68	0.73	0.75	0.80	0.80
L-Lys	0.44	0.44	0.44	0.44	0.44	0.44
DL-Met	0.18	0.19	0.19	0.19	0.20	0.20
L-Thr	0.22	0.22	0.23	0.23	0.24	0.24
L-Trp	0.08	0.08	0.07	0.08	0.08	0.08
L-Val	0.06	0.07	0.07	0.08	0.08	0.08
Fumaric acid	0.50	0.38	0.25	0.13	---	---
Formic acid[5]	0.48	0.36	0.24	0.12	---	---
ZnO	---	---	---	---	---	0.40
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
Phytase[6]	0.06	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100	100
Calculated analysis						
SID amino acids, %						
Lys	1.36	1.36	1.36	1.36	1.36	1.36
Ile:Lys	57	57	57	57	57	57

Leu:Lys	118	116	115	114	113	113
Met:Lys	34	34	34	34	34	34
Met and Cys:Lys	56	56	56	56	56	56
Thr:Lys	66	66	66	66	66	66
Trp:Lys	22.2	22.2	22.3	22.4	22.4	22.4
Val:Lys	70	70	70	70	70	70
His:Lys	36	36	36	36	36	36
Total Lys, %	1.51	1.52	1.52	1.52	1.52	1.52
NE, kcal/kg	2,606	2,610	2,610	2,615	2,617	2,606
SID Lys:NE, g/Mcal	5.22	5.21	5.20	5.20	5.20	5.22
CP, %	21.2	21.2	21.1	21.1	21.1	21.1
Ca, %	0.50	0.50	0.50	0.51	0.51	0.51
P, %	0.52	0.52	0.53	0.53	0.54	0.54
STTD P, %	0.46	0.46	0.46	0.46	0.46	0.46
Analyzed ABC-4, meq/kg	113	173	233	280	333	393

[1] Phase 1 diets were fed from d 0 to 10 post-weaning.

[2] Diet contained 3,000 mg/kg of Zn from ZnO.

[3] AX3 Digest; Protekta; Newport Beach, CA.

[4] HP 300; Hamlet Protein; Findlay, OH.

[5] Amasil NA, BASF, Florham Park, NJ.

[6] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 1,250 FYT/kg.

Table 2.3 Phase 2 diet composition (as-fed basis), Exp. 1[1]

	Calculated ABC-4, meq/kg:					
Item:	200	247	296	343	390	445[2]
Ingredients, %						
Corn	58.70	58.44	58.12	57.85	57.53	57.28
Soybean meal	18.00	18.00	18.00	18.00	18.00	18.00
Crystalline lactose	7.50	7.50	7.50	7.50	7.50	7.50
Specialty soy protein concentrate[3]	10.00	7.50	5.00	2.50	---	---
Enzymatically treated soybean meal[4]	---	3.00	6.05	9.05	12.10	12.10
Corn oil	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.51	0.51	0.52	0.53	0.53	0.53
Monocalcium phosphate	1.05	1.00	0.98	0.95	0.93	0.93
Salt	0.68	0.73	0.75	0.78	0.80	0.80
L-Lys	0.52	0.52	0.52	0.52	0.52	0.52
DL-Met	0.20	0.20	0.20	0.21	0.21	0.21
L-Thr	0.22	0.22	0.22	0.22	0.23	0.23
L-Trp	0.09	0.09	0.09	0.09	0.09	0.09
L-Val	0.12	0.12	0.13	0.13	0.13	0.13
Fumaric acid	0.50	0.38	0.25	0.13	---	---
Formic acid[5]	0.48	0.36	0.24	0.12	---	---
ZnO	---	---	---	---	---	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
Phytase[6]	0.06	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100	100
Calculated analysis						
SID amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	57	57	57	57	57	57
Leu:Lys	116	115	114	113	112	112

Met:Lys	36	36	36	36	36	36
Met and Cys:Lys	56	56	56	56	56	56
Thr:Lys	63	63	63	63	63	63
Trp:Lys	22.5	22.5	22.2	22.3	22.3	22.3
Val:Lys	71	71	71	71	71	71
His:Lys	36	36	36	36	36	36
Total Lys, %	1.50	1.50	1.50	1.50	1.50	1.50
NE, kcal/kg	2,509	2,515	2,518	2,522	2,524	2,518
SID Lys:NE, g/Mcal	5.38	5.37	5.36	5.35	5.35	5.36
CP, %	21.1	21.1	21.1	21.1	21.0	21.0
Ca, %	0.59	0.58	0.59	0.59	0.60	0.60
P, %	0.56	0.55	0.56	0.56	0.57	0.57
STTD P, %	0.46	0.46	0.46	0.46	0.46	0.46
Analyzed ABC-4, meq/kg	133	187	233	300	340	373

[1] Phase 2 diets were fed from d 10 to 23 post-weaning.

[2] Diet contained 2,000 mg/kg of Zn from ZnO.

[3] AX3 Digest; Protekta; Newport Beach, CA.

[4] HP 300; Hamlet Protein; Findlay, OH.

[5] Amasil NA, BASF, Florham Park, NJ.

[6] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 1,250 FYT/kg.

Table 2.4 Phase 1 diet composition (as-fed basis), Exp. 2[1]

		Soy protein replacement	Acidifier removal		
Calculated ABC-4, meq/kg:	200	290	271	362	449[2]
Ingredients, %					
Corn	50.65	48.52	51.37	49.27	48.87
Soybean meal	16.31	16.31	16.31	16.31	16.31
Crystalline lactose	15.00	15.00	15.00	15.00	15.00
Specialty soy protein concentrate[3]	9.38	---	9.38	---	---
Enzymatically treated soybean meal[4]	---	11.40	---	11.40	11.40
Spray-dried bovine plasma	2.50	2.50	2.50	2.50	2.50
Corn oil	2.00	2.00	2.00	2.00	2.00
Limestone	0.35	0.38	0.35	0.38	0.38
Monocalcium phosphate	0.93	0.85	0.93	0.83	0.83
Salt	0.70	0.83	0.70	0.83	0.83
L-Lys	0.44	0.44	0.44	0.44	0.44
DL-Met	0.19	0.19	0.19	0.20	0.20
L-Thr	0.22	0.24	0.23	0.24	0.24
L-Trp	0.08	0.08	0.08	0.08	0.08
L-Val	0.08	0.09	0.09	0.09	0.09
Fumaric acid	0.38	0.38	---	---	---
Formic acid[5]	0.36	0.36	---	---	---
ZnO	---	---	---	---	0.40
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Phytase[6]	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100
Calculated analysis					
SID amino acids, %					
Lys	1.36	1.36	1.36	1.36	1.36

Ile:Lys	56	56	56	56	56
Leu:Lys	116	112	116	112	112
Met:Lys	34	34	34	34	34
Met and Cys:Lys	56	56	56	56	56
Thr:Lys	66	66	66	66	66
Trp:Lys	22.2	22.4	22.2	22.4	22.4
Val:Lys	70	70	70	70	70
His:Lys	36	36	36	36	36
Total Lys, %	1.51	1.52	1.51	1.52	1.52
NE, kcal/kg	2,599	2,586	2,619	2,606	2,595
SID Lys:NE, g/Mcal	5.23	5.26	5.20	5.22	5.24
CP, %	21.1	20.9	21.2	21.0	21.0
Ca, %	0.50	0.52	0.50	0.51	0.51
P, %	0.53	0.54	0.53	0.54	0.54
STTD P, %	0.46	0.46	0.46	0.46	0.46
Analyzed ABC-4, meq/kg	167	260	253	340	387

[1] Phase 1 diets were fed from d 0 to 10 post-weaning.

[2] Diet contained 3,000 mg/kg of Zn from ZnO.

[3] AX3 Digest; Protekta; Newport Beach, CA.

[4] HP 300; Hamlet Protein; Findlay, OH.

[5] Amasil NA, BASF, Florham Park, NJ.

[6] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.14% STTD P with 1,486 FYT/kg.

Table 2.5 Phase 2 diet composition (as-fed basis), Exp. 2[1]

		Soy protein replacement	Acidifier removal		
Calculated ABC-4, meq/kg:	250	322	321	394	448[2]
Ingredients, %					
Corn	57.11	55.33	57.85	56.07	55.82
Soybean meal	22.41	22.41	22.41	22.41	22.41
Crystalline lactose	7.50	7.50	7.50	7.50	7.50
Specialty soy protein concentrate[3]	7.50	---	7.50	---	---
Enzymatically treated soybean meal[4]	---	9.20	---	9.20	9.20
Corn oil	1.00	1.00	1.00	1.00	1.00
Limestone	0.51	0.53	0.51	0.53	0.53
Monocalcium phosphate	1.00	0.93	1.00	0.93	0.93
Salt	0.70	0.80	0.70	0.80	0.80
L-Lys	0.49	0.49	0.49	0.49	0.49
DL-Met	0.19	0.20	0.19	0.20	0.20
L-Thr	0.21	0.21	0.21	0.21	0.21
L-Trp	0.09	0.09	0.09	0.09	0.09
L-Val	0.12	0.13	0.12	0.13	0.13
Fumaric acid	0.38	0.38	---	---	---
Formic acid	0.36	0.36	---	---	---
ZnO	---	---	---	---	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Phytase[6]	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100
Calculated analysis					
SID amino acids, %					
Lys	1.35	1.35	1.35	1.35	1.35
Ile:Lys	58	58	58	58	58

Leu:Lys	116	113	116	113	113
Met:Lys	35	35	35	35	35
Met and Cys:Lys	56	56	56	56	56
Thr:Lys	63	63	63	63	63
Trp:Lys	22.6	22.7	22.6	22.7	22.7
Val:Lys	71	71	71	71	71
His:Lys	36	36	36	36	36
Total Lys, %	1.50	1.50	1.50	1.50	1.50
NE, kcal/kg	2,500	2,491	2,520	2,511	2,504
SID Lys:NE, g/Mcal	5.40	5.43	5.36	5.39	5.40
CP, %	21.4	21.3	21.4	21.4	21.3
Ca, %	0.59	0.60	0.59	0.60	0.60
P, %	0.56	0.57	0.56	0.57	0.57
STTD P, %	0.46	0.46	0.46	0.46	0.46
Analyzed ABC-4, meq/kg	227	300	297	365	397

[1] Phase 2 diets were fed from d 10 to 24 post-weaning.

[2] Diet contained 2,000 mg/kg of Zn from ZnO.

[3] AX3 Digest; Protekta; Newport Beach, CA.

[4] HP 300; Hamlet Protein; Findlay, OH.

[5] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.14% STTD P with 1,486 FYT/kg.

Table 2.6 Effects of increasing diet acid-binding capacity-4 (ABC-4) on nursery pig performance and fecal dry matter (DM), Exp. 1[1]

Phase 1 ABC-4, meq/kg:	150	203	256	311	365	425		*P* =		
Phase 2 ABC-4, meq/kg:	200	247	296	343	390	445		ABC-4[3]		
ZnO:	-	-	-	-	-	+[2]	SEM	Linear	Quadratic	ZnO[4]
BW, kg										
d 0	5.9	5.9	5.9	5.9	5.9	5.9	0.16	0.494	0.945	0.604
d 10	6.9*	7.1	7.0	7.0*	7.0*	7.3	0.20	0.490	0.198	0.011
d 23	11.9	12.3	12.4	11.8*	11.8*	12.5	0.25	0.105	0.014	0.004
d 38	21.2	22.1	22.1	21.4	21.1	21.8	0.45	0.347	0.007	0.136
Phase 1 (d 0 to 10)										
ADG, g	98*	122	116	112*	114*	145	8.2	0.396	0.163	0.009
ADFI, g	109*	124	125	120	123	140	5.7	0.195	0.156	0.034
G:F, g/kg	883	981	884	887	910	993	5.6	0.812	0.778	0.228
Phase 2 (d 10 to 23)										
ADG, g	390	402	407	371	365	397	10.1	0.013	0.045	0.027
ADFI, g	506	530	534	510	500	539	12.3	0.415	0.037	0.391
G:F, g/kg	772	758	759	726	729	734	11.9	0.001	0.944	0.722
Experimental period (d 0 to 23)										
ADG, g	262*	279	277	258*	256*	287	6.9	0.144	0.030	0.002
ADFI, g	331*	351	352	340	336*	365	7.9	0.921	0.042	0.009
G:F, g/kg	790	794	788	759	762	786	9.0	0.002	0.432	0.060
Common period (d 23 to 38)										

ADG, g	618	651	647	637	615	619	14.6	0.588	0.012	0.807
ADFI, g	855	889	877	883	862	862	17.8	0.877	0.110	0.969
G:F, g/kg	723	733	737	721	712	716	16.2	0.178	0.043	0.588
Overall (d 0 to 38)										
ADG, g	401	425	421	408	397	418	8.0	0.253	0.004	0.180
ADFI, g	537	562	557	554	543	562	9.8	0.884	0.046	0.157
G:F, g/kg	748	756	758	736	731	745	6.0	0.003	0.022	0.202
Fecal DM, %[5]										
d 10	22.6	21.8	19.7	19.2	19.5	21.1	1.24	0.001	0.243	0.190
d 17	24.5	25.2*	25.4*	23.3	22.2	22.2		0.022	0.080	0.964
d 23	26.5	26.9	26.5	25.5	26.8	25.5		0.759	0.786	0.333

* Means within a row differ from the high ABC-4 diet with ZnO ($P < 0.05$). Dunnett's test with pigs fed the high ABC-4 diet with ZnO serving as the control.

[1] A total of 360 pigs (initial BW of 5.9 ± 0.16 kg) across two barns were used in a 38-d nursery trial. A total of 6 dietary treatments were utilized with increasing acid-binding capacity-4 (ABC-4) plus a control diet. Increasing the ABC-4 level of the diet was accomplished by replacing specialty soybean meal (-13 meq/kg; AX3 Digest; Protekta; Newport Beach, CA) with enzymatically treated soybean meal (753 meq/kg; HP 300; Hamlet Protein; Findlay, OH) and decreasing the levels of organic acids (fumaric acid and formic acid).

[2] Formulated the same as the highest ABC-4 level, but with the addition of 3,000 and 2,000 mg/kg of Zn from ZnO in phase 1 and 2, respectively, which further increased the ABC-4 value.

[3] Linear and quadratic effects compared increasing ABC-4 levels in diets without ZnO. The single diet containing ZnO is excluded from linear and quadratic analysis.

[4] Comparison of the two highest ABC-4 levels with or without the addition of ZnO. Diets with ABC-4 values below 365 mg/kg were excluded from analysis.

[5] Treatment × day, $P = 0.237$; Treatment, $P = 0.021$; Day, $P < 0.001$. The P-values represented in the data table show the effect of treatment within day.

Table 2.7 Effects of increasing the diet acid-binding capacity-4 (ABC-4) with specialty soy protein sources or acidifiers on weanling pig performance and fecal dry matter (DM), Exp. 2[1]

		Replacing soy protein[2]	Acidifier removal[3]							
Phase 1 ABC-4, meq/kg:	200	290	271	362	449		*P* =			
Phase 2 ABC-4, meq/kg:	250	322	321	394	448		ABC-4[4]		Intermediate	
ZnO	-	-	-	-	+	SEM	Linear	Quadratic	method[5]	ZnO[6]
BW, kg										
d 0	6.0	6.0	6.0	6.0	6.0	0.02	0.739	0.579	0.807	0.608
d 10	7.7	7.8	7.7	7.7	7.8	0.12	0.653	0.747	0.410	0.433
d 24	13.6	13.3	13.2	12.8*	14.0	0.31	0.064	0.812	0.798	0.005
d 38	22.6	22.6	22.4	21.8	22.2	0.38	0.154	0.508	0.714	0.439
Phase 1 (d 0 to 10)										
ADG, g	176	181	169	169	181	11.9	0.687	0.823	0.456	0.476
ADFI, g	191	199	191	189	193	9.9	0.897	0.609	0.552	0.780
G:F, g/kg	913	905	880	875	929	26.0	0.225	0.927	0.415	0.085
Phase 2 (d 10 to 24)										
ADG, g	418	394	392	369*	443	17.4	0.026	0.973	0.907	0.001
ADFI, g	504	481	475	463*	524	22.0	0.098	0.762	0.830	0.014
G:F, g/kg	830	820	825	801	843	13.1	0.130	0.608	0.766	0.030
Experimental period (d 0 to 24)										

ADG, g	317	305	297	286*	334	13.7	0.062	0.978	0.631	0.005
ADFI, g	373	363	355	349	386	15.7	0.187	0.862	0.675	0.047
G:F, g/kg	849	840	837	820*	862	8.8	0.024	0.655	0.838	0.001
Common period (d 24 to 38)										
ADG, g	643*	658*	652*	641*	588	20.5	0.949	0.310	0.712	0.005
ADFI, g	900	963*	943	912	882	22.3	0.694	0.024	0.488	0.307
G:F, g/kg	714*	684	691	706*	668	12.7	0.510	0.021	0.609	0.006
Overall (d 0 to 38)										
ADG, g	437	435	427	417	427	9.9	0.159	0.695	0.579	0.454
ADFI, g	568	583	570	556	569	13.9	0.564	0.291	0.510	0.516
G:F, g/kg	771	746	749	751	751	7.4	0.046	0.058	0.734	0.943
Fecal DM, %[7]										
d 10	23.3	22.1	18.4*	19.5*	25.0	1.25	0.002	0.181	0.003	<0.001
d 17	23.6	22.7	21.9	21.7	23.9		0.126	0.670	0.512	0.077
d 24	24.7	23.9	23.6	21.2	23.1		0.005	0.377	0.810	0.120

* Means within a row differ from the high ABC-4 diet with ZnO ($P < 0.05$). Dunnett's test with pigs fed the high ABC-4 diet with ZnO serving as the control.

[1] A total of 300 pigs (initial BW of 6.0 ± 0.02 kg) were used in a 38-d nursery trial. A total of 5 dietary treatments were utilized with increasing level of acid-binding capacity-4 plus a control diet.

[2] Increasing the ABC-4 level of the diet was accomplished by replacing specialty soybean meal (-13 meq/kg; AX3 Digest; Protekta; Newport Beach, CA) with enzymatically treated soybean meal (797 meq/kg; HP 300; Hamlet Protein; Findlay, OH) on an equal DIS Lys basis.

[3] Increasing the ABC-4 level of the diet was accomplished by removing fumaric and formic acid.

[4] Combines the two intermediate ABC-4 levels and excludes the diet containing ZnO.

[5] Compares the two intermediate ABC-4 levels and excludes all other treatments.

[6] Compares the two high ABC-4 diets while excluding all other treatments.

[7] Treatment × day, $P = 0.022$; Treatment, $P < 0.001$; Day, $P < 0.012$. The P-values represented in the data table show the effect of treatment within day.

Chapter 3 - Effects of dietary acidifiers and other low acid-binding capacity formulation strategies on weanling pig growth performance and fecal dry matter

Abstract

Two experiments were conducted to determine effects of dietary acidifiers and low acid-binding capacity at a pH of 4 (ABC-4) formulation strategies on weanling pig performance and fecal dry matter (DM). In Exp. 1, 300 pigs, initially 6.1 kg, were allotted to 1 of 6 dietary treatments fed in two phases with 5 pigs per pen and 10 replications per treatment. Treatment diets were formulated using four acidifiers to target an ABC-4 of 200 and 250 meq/kg in phases 1 and 2, respectively. The acidifiers included fumaric acid (Primary Products Ingredients Americas LLC, Decatur, IL), Activate DA (Novus, St. Charles, MO), KEM-GEST (Kemin, Des Moines, IA), and ACID-AID (Alltech, Nicholasville, KY) added at 0.36, 0.87, 1.01, and 0.84% of the diet, respectively. The fifth treatment did not contain acidifiers which increased the ABC-4 value of the diet by 40 meq/kg. The last treatment was the same formulation as the diet without acidifiers, but with the addition of pharmacological levels of Zn from ZnO. From d 0 to 24 and d 0 to 38, pigs fed low ABC-4 diets had increased ($P \leq 0.020$) G:F compared to pigs fed the high ABC-4 diet. In Exp. 2, 725 pigs, initially 5.9 kg, were allotted to 1 of 5 dietary treatments fed in two phases with 5 pigs per pen and 29 replications per treatment. Three treatments were formulated using 0.46% fumaric acid with an additional low ABC-4 formulation strategy to target the same low ABC-4 level as Exp. 1. The three strategies consisted of low Ca levels, 0.6% formic acid (Amasil NA; BASF; Florham Park, NJ), or replacing whey permeate with crystalline lactose. The fourth treatment was formulated to be 100 meq/kg greater than the low ABC-4 diets.

The final treatment was the same formulation as the high ABC-4 diet but with the addition of pharmacological levels of Zn. From d 0 to 24 and d 0 to 38, pigs fed the crystalline lactose diet had decreased ($P < 0.05$) ADFI compared to the other low ABC-4 formulation strategies. Pigs fed low ABC-4 diets had increased ($P \leq 0.024$) G:F and fecal DM compared to the 100 meq/kg higher ABC-4 diet. From d 0 to 24, pigs fed the diet containing ZnO had increased ($P \leq 0.001$) ADG and ADFI compared to the high ABC-4 diet without ZnO. In summary, the combination of fumaric and formic acid had the best performance among low ABC-4 formulation strategies, with pigs fed low ABC-4 diets having improved feed efficiency and fecal DM compared to a higher ABC-4 diet when the diets did not contain ZnO.

Key Words: Acid-binding capacity, acidifiers, calcium, fecal dry matter, lactose, weanling pigs

Introduction

Dietary acidifiers are commonly used in weanling pig diets as a method to reduce pH in the gastrointestinal tract promoting nutrient digestibility and inhibiting pathogenic bacteria (Pluske et al., 2021). However, dietary acidifiers are often added without considering the effect other ingredients have on the pH of the gastrointestinal tract. Low acid-binding capacity formulated diets consider the impact of ingredient combinations in the diet on weanling pig stomach pH opposed to the effect of an individual ingredient. Specifically, acid-binding capacity at a pH of 4 (ABC-4) is the amount of hydrochloric acid required to achieve a stable pH of 4 for 1 kg of ingredient or diet (Lawlor et al., 2005; Stas et al., 2022). Previous research has observed improvements in growth performance and fecal dry matter (DM) when diets are formulated to 200 meq/kg from d 0 to 10 post-weaning followed by 250 meq/kg from d 10 to 24 post-weaning (Stas et al., 2024). The use of dietary acidifiers is a simple strategy that can be incorporated to

lower the overall ABC-4 value of the complete diet without affecting other nutrients or ingredients (Stas et al., 2022). However, dietary acidifiers differ in their individual ingredient ABC-4 values because of different characteristics such as inorganic vs. organic origins, differences in pKa values, commercial products that are based on acid blends, or final product presentation as an acid salt. Therefore, different dietary acidifiers will likely have different effects on the stomach acidity of young pigs if they are incorporated at the same rate (Lawlor et al., 2005; Wang et al., 2023).

There has been extensive research on the use of dietary acidifiers and overall, it appears to be positively correlated with growth performance (Wang et al., 2022). However, there is little research evaluating acidifiers in combination with low ABC-4 formulation strategies. A review by Kil et al. (2011) reported that although dietary acidifiers aid in reducing the pH of the stomach in weanling pigs, the reduction in pH is often not large enough to improve nutrient digestion and prevent proliferation of pathogenic organisms. This observation may be due to other ingredients within the diet that are contributing to an elevated stomach pH and thus limiting dietary acidifier's effectiveness. Using dietary acidifiers in combination with low ABC-4 formulation strategies may allow better acidification of the gastrointestinal tract to improve growth performance and lower the occurrence of post-weaning diarrhea. In addition to dietary acidifiers, there are various strategies that can be used to lower the ABC-4 level of the diet. Lowering the Ca level of the diet by reducing the amount of calcium carbonate has been observed to increase growth performance in weanling pigs (Wu et al., 2018; Warner et al., 2023). The use of specialty protein sources and lactose sources that have low ABC-4 values has been observed to improve feed efficiency in weanling pigs (Zhao et al., 2021; Stas et al., 2024). Using multiple acidifiers with low ABC-4 values can have synergistic effects because of their different

pKa values as well as their combined ability to further reduce the ABC-4 (Nguyen et al., 2020). Evaluation of dietary acidifiers and formulation strategies to target the same low ABC-4 level is warranted to determine if the response is altered in newly weaned pigs.

Therefore, the objective of these studies was to determine the effects of different dietary acidifiers and low ABC-4 formulation strategies on weanling pig performance and fecal DM. Our hypothesis was that different dietary acidifiers and low ABC-4 formulation strategies will elicit a similar response when used to target the same ABC-4 level of 200 meq/kg from d 0 to 10 post-weaning and 250 meq/kg from d 10 to 24 post-weaning resulting in improved weanling pig performance and fecal DM compared to higher ABC-4 levels.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Experiment 1 was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The study was conducted in two identical nursery rooms. Experiment 2 was conducted in two research facilities, the Kansas State University Swine Teaching and Research Center and the Kansas State University Segregated Early Weaning facility, both located in Manhattan, KS. The first facility used in Exp. 2 was a single nursery room and the second facility was two identical nursery barns. All facilities in both experiments were completely enclosed, environmentally controlled, and mechanically ventilated. Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide *ad libitum* access to feed and water. Pens (1.2 × 1.2 m) had tri-bar floors and allowed approximately 0.30 m^2/pig.

ABC-4 analysis. A sample of each ingredient and complete diet were analyzed for ABC-4 at the Kansas State University Swine Nutrition Laboratory in Manhattan, KS (Table 1).

Analysis was accomplished by the procedure outlined by Stas et al. (2022). Each ingredient and diet were analyzed in triplicate and the average of each analysis was used for the overall analyzed ABC-4 value. Individual ingredient ABC-4 values were used for experimental diet formulation. Complete diets were analyzed to confirm dietary ABC-4 formulation from ingredient values.

Experiment 1

Animals and treatment structure. The objective of Exp. 1 was to determine the effects of various commercial dietary acidifiers formulated to an equal ABC-4 level on nursery pig performance and fecal DM. A total of 300 weanling pigs (DNA 241 × 600; initially 6.1 ± 0.02 kg BW) were used in a 38-d trial. At weaning, pens of pigs were randomly allotted to 1 of 6 dietary treatments with 10 replications per treatment (Tables 2 and 3). Dietary treatments were fed in two phases with phase 1 provided from d 0 to 10 post-weaning, followed by phase 2 until d 24 post-weaning. Dietary treatments consisted of four low ABC-4 diets formulated to 200 and 250 meq/kg in phase 1 and 2, respectively. The four low ABC-4 diets utilized four different dietary acidifiers to achieve the low ABC-4 targets. Dietary acidifiers consisted of fumaric acid (Primary Products Ingredients Americas LLC, Decatur, IL), Activate DA (Novus, St. Charles, MO), KEM-GEST (Kemin, Des Moines, IA), and ACID-AID (Alltech, Nicholasville, KY) included at 0.36, 0.87, 1.01, and 0.84% of the diet in both phases, respectively. Fumaric acid, Activate DA, KEM-GEST, and ACID-AID had individual ingredient ABC-4 values of -10,873, -4,487, -3,800, and -4,680 meq/kg, respectively which ultimately determined their inclusion to achieve the target ABC-4 of experimental diets. The level of Activate DA, KEM-GEST, and ACID-AID in the diets exceeded supplier-recommended inclusion rates to achieve the low ABC-4 levels of 200 and 250 meq/kg in phase 1 and 2, respectively. The fifth treatment did not contain any dietary

acidifiers which increased the ABC-4 level by 40 meq/kg in both phases. All five dietary treatments contained 100 mg/kg of Zn provided by the trace mineral premix and did not contain any pharmacological levels of Zn. The sixth and final dietary treatment was the same formulation as the fifth diet containing no acidifiers, but with the addition of 3,000 and 2,000 mg/kg of Zn from ZnO in phases 1 and 2, respectively. The addition of ZnO increased the diet ABC-4 value by 87 and 54 meq/kg in phases 1 and 2, respectively. The basal diets for phases 1 and 2 were manufactured at Hubbard feeds in Beloit, KS. The basal diets were divided into 6 batches and treatment ingredients were added and mixed at the Kansas State University O.H. Kruse Feed and Technology Innovation Center in Manhattan, KS to form the six experimental diets.

Individual pig weights and feed disappearance were measured on d 10 and weekly afterwards to determine ADG, ADFI, and G:F. Fecal samples were collected on d 10, 17, and 24 to determine percentage fecal DM from the same three medium weight pigs from each pen. After collection, fecal samples were dried at 55°C in a forced air oven for 48 h and the ratio of dried to wet fecal weight determined percentage fecal DM.

Experiment 2

Animals and treatment structure. The objective of Exp. 2 was to determine the effect of dietary acidifiers and other low ABC-4 formulation strategies on weanling pig performance and fecal DM. A total of 725 pigs (DNA 200 × 400; DNA 241 × 600; initially 5.9 ± 0.09 kg BW) were used in a 38-d trial across two research facilities. At weaning, pens of pigs were randomly allotted to 1 of 5 dietary treatments with 29 replications per treatment. Dietary treatments were fed in two phases with phase 1 fed according to a feed budget of 2.3 kg/pig, followed by phase 2 diets which were fed until d 24 post-weaning. Dietary treatments consisted of three low ABC-4

diets formulated to 200 and 250 meq/kg in phases 1 and 2, respectively. The three low ABC-4 diets used 0.46% fumaric acid in combination with three other strategies to achieve the low ABC-4 level. The first strategy to lower the ABC-4 of the diet consisted of low Ca levels by decreasing the amount of limestone in the diet. Limestone has an individual ingredient ABC-4 value of 18,384 meq/kg. The low Ca treatment was formulated to have a Ca:P ratio 0.20 lower than all other dietary treatments. The second strategy utilized 0.6% formic acid (Amasil NA, BASF, Florham Park, NJ) in combination with the fumaric acid. The third strategy to lower ABC-4 consisted of replacing whey permeate (Dairylac 80, International Ingredients Corporation, Fenton, MO) with crystalline lactose based on lactose equivalency. Whey permeate and crystalline lactose have individual ingredient ABC-4 values of 433 and 53 meq/kg, respectively. This strategy led to an ABC-4 of 193 and 262 meq/kg in phases 1 and 2, respectively. Ultimately, a complete replacement of whey permeate with crystalline lactose resulted in an ABC-4 diet slightly below target for phase 1 and slightly above target for phase 2. However, the average ABC-4 value across the two phases is similar to the other low ABC-4 formulation strategies. The alternative lactose strategy also adjusted the inclusion of microbially enhanced soybean meal (MEPRO; Prairie Aquatech; Brooking, SD) to maintain the same level of soybean meal across all diets. The fourth dietary treatment was formulated to an ABC-4 level of 300 and 350 meq/kg in phases 1 and 2, respectively. This diet did not contain any acidifiers, utilized only whey permeate as the lactose source, and had a Ca:P ratio of 0.9:1 and 1.0:1 in phases 1 and 2, respectively. All four dietary treatments contained 110 mg/kg of Zn from the trace mineral premix. The fifth and final dietary treatment was the same as the fourth diet, but with the addition of 3,000 and 2,000 mg/kg of Zn from ZnO in phases 1 and 2, respectively. The addition of ZnO increased the ABC-4 value by 87 and 54 meq/kg in phases 1 and 2, respectively.

Diets were manufactured at the Kansas State University O.H. Kruse Feed and Technology Innovation Center in Manhattan, KS. Individual pig weights and feed disappearance were measured on d 8, 17, 24, 31, and 38 to determine ADG, ADFI, and G:F. Fecal samples were collected on d 8, 17, and 24 to determine fecal DM percentage and followed the same procedure outlined for Exp. 1.

In both experiments, phase 1 and 2 diets were formulated to contain 1.35% standardized ileal digestible (SID) Lys. Following phase 2, all pigs were fed a common corn-soybean meal-based diet until the completion of the study on d 38. The common diet was formulated to 1.30% SID Lys and met or exceeded all other nutrient requirements established by the NRC (2012). Phase 1 diets were manufactured in pellet form while phase 2 and 3 diets were manufactured in meal form.

Statistical analysis

For both experiments, data were analyzed as a completely randomized design using the RStudio environment (Version 1.3.1093, RStudio, Inc., Boston, MA) of the R programming language [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria] with pen as the experimental unit. Room/barn in each trial was included as a random effect. Differences between acidifiers used in Exp. 1 was tested by a contrast comparison of the four low ABC-4 diets with different acidifiers and excluded the diets not containing acidifiers. Similarly, the differences between strategies for Exp. 2 was tested by a contrast comparison of the three strategies to lower ABC-4 value of the diet and excluded the two high ABC-4 diets. In both experiments, the ABC-4 level effect was tested by a comparison of the mean of the low ABC-4 diets vs the single high ABC-4 not containing ZnO. The ZnO effect was tested by a pairwise comparison of the high ABC-4 levels with or without ZnO and excluded all

low ABC-4 diets. Fecal DM were analyzed using the fixed effects of day, treatment, and the associated interaction accounting for repeated measures over time. Differences between treatments and day (where appropriate) as well as their interaction were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

ABC-4 analysis

All dietary analyzed ABC-4 values were less than calculated values regardless of treatment, phase, or experiment which is consistent with previous research (Lu et al., 2016; Stas et al., 2022). In Exp. 1, the analyzed ABC-4 values for diets without ZnO were on average 27 and 19 meq/kg lower than calculated values for phases 1 and 2, respectively. Analyzed ABC-4 values for the diet containing ZnO was 60 and 31 meq/kg lower than calculated values for phases 1 and 2, respectively. In Exp. 2, the analyzed ABC-4 values for diets without ZnO were on average 33 and 20 meq/kg lower than calculated values for phases 1 and 2, respectively. Analyzed ABC-4 value for the diet containing ZnO was 74 and 44 meq/kg lower than calculated value in phase 1 and 2, respectively. However, analyzed ABC-4 values still increased or decreased with respect to dietary formulation strategy and were similar among the low ABC-4 treatments within phase in each experiment.

Experiment 1

There were no differences in growth performance between pigs fed the 4 dietary acidifiers for phase 1 (d 0 to 10), phase 2 (d 10 to 24), or the experimental period (d 0 to 24). In the common period (d 24 to 38), there was a tendency observed ($P = 0.094$) with pigs previously fed KEM-GEST having numerically lower G:F. There were no differences in growth

performance overall (d 0 to 38) or for any fecal DM collection period between pigs fed the 4 dietary acidifiers.

There were no performance differences between pigs fed either the low or high ABC-4 diets in phase 1 (d 0 to 10). In phase 2 (d 10 to 24) and the overall experimental diet period (d 0 to 24), pigs fed the low ABC-4 diets had increased ($P \leq 0.020$) G:F compared to pigs fed the 40 meq/kg higher ABC-4 diet. There were no differences between pigs previously fed either ABC-4 level for the common period (d 24 to 38). Overall (d 0 to 38), pigs fed the low ABC-4 levels had increased ($P = 0.013$) G:F compared to pigs fed the 40 meq/kg higher ABC-4 diet. There were no differences between pigs fed either ABC-4 level for all fecal DM collection periods.

There were no differences in growth performance between pigs fed diets with or without ZnO in phase 1 (d 0 to 10) or phase 2 (d 10 to 24). In the experimental period (d 0 to 24), pigs fed diets containing ZnO tended to have increased ($P \leq 0.072$) BW and G:F compared to pigs fed diets without ZnO. There were no differences between pigs fed diets with or without ZnO for the common period (d 24 to 38), overall (d 0 to 38), or for any fecal DM collection periods.

Experiment 2

There were no differences in growth performance between pigs fed the three low ABC-4 formulation strategies for phase 1 (d 0 to 8). In phase 2 (d 10 to 24) and the experimental period (d 0 to 24), pigs fed low Ca or the combination of acidifier strategies had increased ($P < 0.05$) ADFI compared to pigs fed the crystalline lactose diet. In the common period (d 24 to 38) and overall (d 0 to 38), there was a tendency observed ($P \leq 0.094$) with pigs fed the combination of acidifiers strategy having the greatest BW and ADG. Overall (d 0 to 38), pigs fed low Ca or the combination of acidifiers strategies had increased ($P < 0.05$) ADFI compared to pigs fed the

crystalline lactose diet. On d 8, pigs fed the crystalline lactose diet had increased ($P < 0.05$) fecal DM compared to the combination of acidifiers strategy with the low Ca strategy intermediate.

In phase 1 (d 0 to 8), pigs fed the diets with low ABC-4 levels had increased ($P = 0.006$) G:F and tended to have increased ($P \leq 0.099$) BW and ADG compared to pigs fed the high ABC-4 diet. There were no differences between pigs fed either ABC-4 level for phase 2 (d 8 to 24). In the experimental period (d 0 to 24), pigs fed the low ABC-4 levels had increased ($P = 0.022$) G:F compared to pigs fed the high ABC-4 diet. In the common period (d 24 to 38), pigs previously fed low ABC-4 levels tended to have increased ($P = 0.062$) G:F compared to pigs fed the high ABC-4 diet. Overall (d 0 to 38), pigs fed the low ABC-4 level had increased ($P = 0.005$) G:F compared to pigs fed the high ABC-4 diet. For all fecal collection periods (d 8, 17, and 24), pigs fed the low ABC-4 levels had increased ($P \leq 0.024$) fecal DM compared to pigs fed the high ABC-4 diet.

For ZnO effects, during phase 1 (d 0 to 8), phase 2 (d 8 to 24), and the experimental period (d 0 to 24), pigs fed diets containing ZnO had increased ($P \leq 0.003$) BW, ADG, and ADFI compared to pigs fed the diet without ZnO. In addition, pigs fed diets containing ZnO had increased ($P = 0.003$) G:F in phase 1 (d 0 to 8) and tended to have increased ($P = 0.054$) G:F in the experimental period (d 0 to 24) compared to pigs fed the diet without ZnO. There were no differences between pigs previously fed diets with or without ZnO in the common period (d 24 to 38). Overall (d 0 to 38), pigs fed diets containing ZnO tended to have increased ($P = 0.068$) ADG compared to pigs fed diets without ZnO. There were no differences in fecal DM for pigs fed diets with or without ZnO for all collection periods.

Discussion

There has been extensive research regarding the use of dietary acidifiers as a method to improve growth performance and gastrointestinal health of weanling pigs. Generally, the use of dietary acidifiers has been proposed to decrease stomach pH which increases gastric pepsin activity, inhibits proliferation of pathogenic bacteria in the gastrointestinal tract, and improves nutrient digestion (Kil et al., 2011; Papatsiros and Billinis, 2012; Liu et al., 2018). Reduced stomach pH and increased gastric pepsin activity can be attributed to the acid-binding capacity characteristics of dietary acidifiers (Lawlor et al., 2005). Acidifiers typically have negative ABC-4 values which aids in reducing the ABC-4 of the complete diet. A negative ABC-4 value indicates the initial pH of acidifiers was below 4 and had to be titrated with sodium hydroxide to raise the pH (Stas et al., 2022). Although all acidifiers used in the current study were observed to have negative ABC-4 values, their individual ABC-4 varied between acidifiers which ultimately determined their inclusion in the diet. Fumaric acid has a 6,550 meq/kg lower ABC-4 value than the mean of the commercial acidifiers (Activate DA, KEM-GEST, and ACID-AID). The commercial acidifiers used in this study were all acid blends and included a carrier such as ground corn cobs. The use of a carrier dilutes the acid and increases the ABC-4 value of the final product. Therefore, in dietary ABC-4 formulation, a high inclusion of the commercial acidifiers is needed to achieve the same reduction in ABC-4 of the diet as a pure acid. Variation in ABC-4 between the three commercial acidifiers (Activate DA, KEM-GEST, and ACID-AID) is likely a result of different organic acids used and proportions of acids that make up each commercial acidifier.

In Exp. 1, a high inclusion rate of the three commercial acidifiers was used to get the same reduction in ABC-4 as the diet containing 0.36% fumaric acid because of the differences in

their individual ingredient ABC-4. High inclusion of dietary acidifiers is often associated with poorer palatability of the diet, thus decreased ADFI. Walsh et al. (2007) and Ahmed et al. (2014) both observed pigs fed diets containing 0.4% of an acidifier blend decreased ADFI compared to diets without acidifiers. In contrast, in Exp. 1 we did not observe any significant differences in ADFI even though the three commercial acidifiers were included at more than double the rate of the previously mentioned studies. However, numerically the three commercial acidifiers decreased ADFI by 5.2% on average compared to the diet without acidifiers during the experimental period. In order to achieve the desired ABC-4 levels of 200 meq/kg from d 0 to 10 post-weaning and 250 meq/kg from d 10 to 24 post-weaning, acidifier blends may need to be included in the diet at an inclusion rate that could negatively impact feed intake. Therefore, other low ABC-4 formulation strategies should be incorporated along with dietary acidifiers to reach the desired ABC-4 level for weanling pigs without decreasing palatability.

In Exp. 2, three different low ABC-4 formulation strategies were used along with a dietary acidifier to achieve the target ABC-4 level. Fumaric acid was selected as the primary acidifier for Exp. 2 based off the results from Exp. 1. Previous research has also shown fumaric acid lowers the gastric pH of pigs when included in the diet (Bosi et al., 1999; Hanczakowska et al., 2011; Grecco et al., 2018). Among the three low ABC-4 formulation strategies, the combination of fumaric and formic acid had the greatest growth rate throughout the study and highest ending BW. This observation may be explained by possible synergistic effects of fumaric and formic acid that has been observed previously with other organic acids (Wei et al., 2021; Xu et al., 2022). However, pigs fed the low Ca and fumaric acid formulation strategy had similar performance to the combination of acidifiers during the experimental period. Previous research conducted by Warner et al. (2023) also observed benefits in weanling pig growth performance

from feeding diets with low calcium carbonate levels and a dietary acidifier. The authors reported additive effects on growth performance from the two low ABC-4 formulation strategies which agrees with the results of the current study. While decreasing the amount of Ca in the diet is a method to decrease the ABC-4 level of the complete diet, another strategy could be using alternative Ca sources with low ABC-4 levels, such as calcium formate, to meet the desired Ca level (Lawlor et al., 2006).

While the lactose replacement strategy did not affect ADG and G:F, using crystalline lactose to decrease the ABC-4 level of the diet resulted in a reduction in ADFI. A similar response was also observed by Nessmith et al. (1997) where pigs fed crystalline lactose had a 10.8% reduction in ADFI compared to pigs fed dried whey powder. The reduced ADFI could be a result of the palatability differences between the two lactose sources. Whey permeate contains easily digestible proteins and oligosaccharides that enhance the quality of the lactose source (Jang et al., 2021; Zhao et al., 2021). Therefore, pigs may have preferred diets containing whey permeate opposed to crystalline lactose because of their nutritional property differences. Although there was a decrease in ADFI, pigs fed crystalline lactose did have increased fecal DM on d 8. Reduced ADFI from pigs fed crystalline lactose may have resulted in less protein to be fermented in the gastrointestinal tract which could have increased the firmness of the feces (Diether and Willing, 2019). However, the main reduction in ADFI was observed when pigs transitioned to phase 2 diets and the increase in fecal DM from pigs fed crystalline lactose was observed on d 8 and not d 17 or 24. Although there were differences observed between the different low ABC-4 formulation strategies, on average, the low ABC-4 formulated diets improved feed efficiency and fecal DM compared to pigs fed higher ABC-4 diets.

In both experiments, the low ABC-4 diets improved feed efficiency compared to the high ABC-4 diets without ZnO. This observation agrees with previous studies formulated to the same low ABC-4 level of 200 and 250 meq/kg in phases 1 and 2, respectively (Stas et al., 2024). The differences in ABC-4 levels between Exp. 1 and 2 explain the variable response to fecal DM. In Exp. 1, there was only a 40 meq/kg difference in ABC-4 levels with no effect on fecal DM. Whereas in Exp. 2, there was a 100 meq/kg difference in ABC-4 of experimental diets with low ABC-4 diets increasing fecal DM. There was also a discrepancy between both experiments for the response to ZnO. Significant improvements in growth performance for diets containing ZnO were only observed in Exp. 2, which is likely a result of the amount of variation between the two experiments. Pigs fed diets containing ZnO had a BW CV of 7.7 and 5.5% for Exp. 1 and 2, respectively. Even though variation was higher with Exp. 1, both experiments had approximately a 10.5% improvement in ADG when ZnO was added to the high ABC-4 diets, although significance was only able to be detected in Exp. 2. Overall, the addition of ZnO improved weanling pig performance as expected.

In summary, the results of both experiments indicate that many strategies and different dietary acidifiers can be used to achieve low ABC-4 diets to improve performance and fecal DM of weanling pigs in diets not containing ZnO. However, depending on the strategy to achieve a low ABC-4 target, the response in pigs may be affected. The use of crystalline lactose decreased feed intake but improved fecal DM shortly after weaning compared to low ABC-4 diets containing whey permeate. The combination of fumaric and formic acid had the greatest ending BW and ADG, but pigs fed diets with low Ca and fumaric acid was not statistically different. Fumaric acid had a much lower ABC-4 value and thus lower inclusion rates are required to achieve the same reduction in dietary ABC-4 as compared with commercial acidifiers.

Commercial acidifiers can still be used to decrease the ABC-4 value of the diet to improve performance and fecal DM, but other strategies will likely need to be incorporated to obtain an ABC-4 level of 200 meq/kg from d 0 to 10 and 250 meq/kg from d 10 to 24 post-weaning. This study helps illustrate how different dietary acidifiers and formulation strategies can be incorporated into weanling pig diets to achieve an ideal ABC-4 level.

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Table 3.1 Analyzed acid-binding capacity at a pH of 4 (ABC-4) of ingredients used in experimental diets[1]

Ingredient, %	Initial pH	ABC-4 (meq/kg)
Corn	6.84	84
Soybean meal	7.04	602
Crystalline lactose	4.61	53
Whey permeate[2]	6.48	433
Microbially enhanced soybean meal[3]	5.11	67
Spray-dried bovine plasma	6.99	713
Fumaric acid[4]	2.31	-10,873
Formic acid[5]	3.02	-8,287
Activate DA[6]	2.99	-4,487
KEM-GEST[7]	2.06	-3,800
ACID-AID[8]	2.21	-4,680
Limestone	9.70	18,384
Monocalcium phosphate	4.22	73
Salt	6.06	15
L-Lys	5.81	83
DL-Met	5.39	137
L-Thr	5.51	160
L-Trp	5.12	120
L-Val	5.52	193
ZnO	9.15	21,863
Vitamin premix with phytase	6.04	3,210
Vitamin premix without phytase	7.07	10,767
Trace mineral premix	5.13	7,867
Phytase	5.95	170

[1] Acid-binding cpacity-4 was determined by the amount of 0.1 N HCl required to lower the initial pH of a sample to a stable pH of 4.00 ± 0.04. Acidifiers were titrated with 0.1 N NaOH to raise the initial pH to a stable pH of 4.00 ± 0.04. Each sample was analyzed in triplicate.

[2] Dairylac 80; International Ingredients Corporation; Fenton, MO.

[3] MEPRO; Prairie Aquatech; Brookings, SD.

[4] Primary Products Ingredients Americas LLC, Decatur, IL

[5] Amasil NA, BASF, Florham Park, NJ.

[6] Novus; St. Charles, MO.

[7] Kemin; Des Moines, IA.

[8] Alltech; Nicholasville, KY.

Table 3.2 Phase 1 diet composition (as-fed basis), Exp. 1[1]

	Dietary acidifier					
	1	2	3	4	-	-
ZnO:	-	-	-	-	-	+
ABC-4, meq/kg:	200	200	200	200	240	327
Ingredients, %						
Corn	54.65	54.14	54.00	54.18	55.01	54.61
Soybean meal	12.00	12.00	12.00	12.00	12.00	12.00
Crystalline lactose	15.00	15.00	15.00	15.00	15.00	15.00
Microbially enhanced soybean meal[2]	10.00	10.00	10.00	10.00	10.00	10.00
Spray-dried bovine plasma	2.50	2.50	2.50	2.50	2.50	2.50
Acidifier 1[3]	0.36	---	---	---	---	---
Acidifier 2[4]	---	0.87	---	---	---	---
Acidifier 3[5]	---	---	1.01	---	---	---
Acidifier 4[6]	---	---	---	0.84	---	---
ZnO	---	---	---	---	---	0.40
Corn oil	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.26	0.26	0.26	0.26	0.26	0.26
Monocalcium phosphate	0.98	0.98	0.98	0.98	0.98	0.98
Salt	0.83	0.83	0.83	0.83	0.83	0.83
L-Lys	0.46	0.46	0.46	0.46	0.46	0.46
DL-Met	0.22	0.22	0.22	0.22	0.22	0.22
L-Thr	0.19	0.19	0.19	0.19	0.19	0.19
L-Trp	0.07	0.07	0.07	0.07	0.07	0.07
L-Val	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix with phytase[7]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100	100
Calculated analysis						
SID amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35

Ile:Lys	55	55	55	55	55	55
Leu:Lys	119	118	118	118	119	119
Met:Lys	35	35	35	35	35	35
Met and Cys:Lys	56	56	56	56	56	56
Thr:Lys	63	63	63	63	63	63
Trp:Lys	20.3	20.3	20.3	20.3	20.3	20.3
Val:Lys	70	70	70	70	70	70
His:Lys	36	36	36	36	36	36
Total Lys, %	1.51	1.51	1.51	1.51	1.51	1.51
NE, kcal/kg	2,619	2,606	2,604	2,608	2,630	2,619
SID Lys:NE, g/Mcal	5.15	5.18	5.18	5.18	5.14	5.16
CP, %	20.3	20.3	20.3	20.3	20.4	20.3
Ca, %	0.46	0.46	0.46	0.46	0.46	0.46
P, %	0.51	0.51	0.51	0.51	0.51	0.51
STTD P, %	0.46	0.46	0.46	0.46	0.46	0.46
Analyzed ABC-4, meq/kg	173	173	173	173	213	267

[1] Phase 1 diets were fed from d 0 to 10 post-weaning.

[2] ME-PRO; Prairie Aquatech; Brookings, SD.

[3] Fumaric acid; Primary Products Ingredients Americas LLC, Decatur, IL

[4] Activate DA; Novus; St. Charles, MO.

[5] KEM-GEST; Kemin; Des Moines, IA.

[6] ACID-AID; Alltech; Nicholasville, KY.

[7] Quantum Blue 5G (ABVista, Plantation, FL) provided an estimated release of 0.13% STTD P with 1,250 FTU/kg.

Table 3.3 Phase 2 diet composition (as-fed basis), Exp. 1[1]

	Dietary acidifier					
	1	2	3	4	-	-
ZnO:	-	-	-	-	-	+
ABC-4, meq/kg:	250	250	250	250	290	344
Ingredients, %						
Corn	60.60	60.09	59.94	60.12	60.96	60.71
Soybean meal	18.75	18.75	18.75	18.75	18.75	18.75
Crystalline lactose	7.50	7.50	7.50	7.50	7.50	7.50
Microbially enhanced soybean meal[2]	8.00	8.00	8.00	8.00	8.00	8.00
Acidifier 1[3]	0.36	---	---	---	---	---
Acidifier 2[4]	---	0.87	---	---	---	---
Acidifier 3[5]	---	---	1.01	---	---	---
Acidifier 4[6]	---	---	---	0.84	---	---
ZnO	---	---	---	---	---	0.25
Corn oil	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	0.41	0.41	0.41	0.41	0.41	0.41
Monocalcium phosphate	1.03	1.03	1.03	1.03	1.03	1.03
Salt	0.80	0.80	0.80	0.80	0.80	0.80
L-Lys	0.52	0.52	0.52	0.52	0.52	0.52
DL-Met	0.23	0.23	0.23	0.23	0.23	0.23
L-Thr	0.22	0.22	0.22	0.22	0.22	0.22
L-Trp	0.08	0.08	0.08	0.08	0.08	0.08
L-Val	0.12	0.12	0.12	0.12	0.12	0.12
Vitamin premix with phytase[7]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15	0.15
Total	100	100	100	100	100	100
Calculated analysis						
SID amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.35	1.35
Ile:Lys	56	56	56	56	56	56

Leu:Lys	117	117	117	117	117	117
Met:Lys	37	37	37	37	37	37
Met and Cys:Lys	56	56	56	56	56	56
Thr:Lys	63	63	63	63	63	63
Trp:Lys	21.0	21.0	21.0	21.0	21.0	21.0
Val:Lys	70	70	70	70	70	70
His:Lys	36	36	36	36	36	36
Total Lys, %	1.50	1.50	1.50	1.50	1.50	1.50
NE, kcal/kg	2,524	2,511	2,507	2,511	2,533	2,526
SID Lys:NE, g/Mcal	5.35	5.38	5.39	5.38	5.34	5.35
CP, %	20.7	20.7	20.7	20.7	20.7	20.7
Ca, %	0.54	0.54	0.54	0.54	0.54	0.54
P, %	0.54	0.54	0.54	0.54	0.54	0.54
STTD P, %	0.46	0.46	0.46	0.46	0.46	0.46
Analyzed ABC-4, meq/kg	233	227	227	227	280	313

[1] Phase 2 diets were fed from d 10 to 24 post-weaning.

[2] ME-PRO; Prairie Aquatech; Brookings, SD.

[3] Fumaric acid; Primary Products Ingredients Americas LLC, Decatur, IL

[4] Activate DA; Novus; St. Charles, MO.

[5] KEM-GEST; Kemin; Des Moines, IA.

[6] ACID-AID; Alltech; Nicholasville, KY.

[7] Quantum Blue 5G (ABVista, Plantation, FL) provided an estimated release of 0.13% STTD P with 1,250 FTU/kg.

Table 3.4 Phase 1 diet composition (as-fed basis), Exp. 2[1]

Fumaric acid:	+	+	+	-	-
Low ABC-4 Strategy:	Low Ca	Formic acid	Crystalline lactose	-	-
ZnO:	-	-	-	-	+
ABC-4, meq/kg:	200	200	193	300	387
Ingredients, %					
Corn	57.65	56.78	59.21	57.84	57.44
Soybean meal	12.00	12.00	12.00	12.00	12.00
Whey permeate[2]	15.00	15.00	---	15.00	15.00
Crystalline lactose	---	---	12.00	---	---
Microbially enhanced soybean meal[3]	7.50	7.50	8.10	7.50	7.50
Spray-dried bovine plasma	2.50	2.50	2.50	2.50	2.50
Fumaric acid[4]	0.46	0.46	0.46	---	---
Formic acid[5]	---	0.60	---	---	---
ZnO	---	---	---	---	0.40
Corn oil	2.00	2.00	2.00	2.00	2.00
Limestone	0.01	0.28	0.28	0.28	0.28
Monocalcium phosphate	0.75	0.75	0.95	0.75	0.75
Salt	0.40	0.40	0.78	0.40	0.40
L-Lys	0.55	0.55	0.55	0.55	0.55
DL-Met	0.24	0.24	0.24	0.24	0.24
L-Thr	0.24	0.24	0.24	0.24	0.24
L-Trp	0.08	0.08	0.09	0.08	0.08
L-Val	0.16	0.16	0.16	0.16	0.16
L-Ile	0.02	0.02	0.02	0.02	0.02
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Phytase[6]	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100
Calculated analysis					
SID amino acids, %					

Lys	1.35	1.35	1.35	1.35	1.35
Ile:Lys	52	52	53	52	52
Leu:Lys	112	111	113	112	112
Met:Lys	36	36	36	36	36
Met and Cys:Lys	56	56	56	57	57
Thr:Lys	64	64	64	64	64
Trp:Lys	21.1	21.1	20.8	21.1	21.1
Val:Lys	70	70	70	70	70
His:Lys	33	33	34	33	33
Total Lys, %	1.50	1.50	1.50	1.50	1.50
NE, kcal/kg	2,657	2,632	2,624	2,661	2,650
SID Lys:NE, g/Mcal	5.08	5.12	5.14	5.07	5.07
CP, %	19.4	19.3	19.5	19.4	19.4
Ca, %	0.36	0.46	0.46	0.36	0.36
P, %	0.51	0.51	0.51	0.51	0.51
STTD P, %	0.46	0.46	0.46	0.46	0.46
Ca:P	0.70	0.90	0.90	0.70	0.70
Na, %	0.40	0.40	0.40	0.40	0.40
Analyzed ABC-4, meq/kg	170	167	163	263	313

[1] Phase 1 diets were according to a feed budget of 2.3 kg/pig.

[2] Dairylac 80; International Ingredients Corporation; Fenton, MO.

[3] MEPRO; Prairie Aquatech; Brookings, SD.

[4] Primary Products Ingredients Americas LLC, Decatur, IL

[5] Amasil NA; BASF; Florham Park, NJ.

[6] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.14% STTD P with 1,486 FYT/kg.

Table 3.5 Phase 2 diet composition (as-fed basis) Exp.2[1]

Fumaric acid	+	+	+	-	-
Low ABC-4 Strategy:	Low Ca	Formic acid	Crystalline lactose	-	-
ZnO:	-	-	-	-	+
ABC-4, meq/kg:	250	250	262	350	404
Ingredients, %					
Corn	57.61	56.74	58.53	57.80	57.55
Soybean meal	22.70	22.70	22.70	22.70	22.70
Whey permeate[2]	10.00	10.00	---	10.00	10.00
Crystalline lactose	---	---	8.00	---	---
Microbially enhanced soybean meal[3]	5.00	5.00	5.40	5.00	5.00
Fumaric acid[4]	0.46	0.46	0.46	---	---
Formic acid[5]	---	0.60	---	---	---
ZnO	---	---	---	---	0.25
Corn oil	1.00	1.00	1.00	1.00	1.00
Limestone	0.15	0.42	0.42	0.42	0.42
Monocalcium phosphate	0.85	0.85	1.00	0.85	0.85
Salt	0.55	0.55	0.80	0.55	0.55
L-Lys	0.53	0.53	0.53	0.53	0.53
DL-Met	0.23	0.23	0.23	0.23	0.23
L-Thr	0.24	0.24	0.24	0.24	0.24
L-Trp	0.08	0.08	0.08	0.08	0.08
L-Val	0.15	0.15	0.15	0.15	0.15
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Trace mineral premix	0.15	0.15	0.15	0.15	0.15
Phytase[6]	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100
Calculated analysis					
SID amino acids, %					
Lys	1.35	1.35	1.35	1.35	1.35
Ile:Lys	55	55	55	55	55

Leu:Lys	113	113	114	113	113
Met:Lys	37	37	37	37	37
Met and Cys:Lys	56	56	56	56	56
Thr:Lys	64	64	64	64	64
Trp:Lys	21.9	21.8	21.9	21.9	21.9
Val:Lys	70	70	70	70	70
His:Lys	35	35	36	35	35
Total Lys, %	1.49	1.49	1.49	1.50	1.50
NE, kcal/kg	2,535	2,513	2,511	2,542	2,535
SID Lys:NE, g/Mcal	5.32	5.37	5.37	5.37	5.37
CP, %	20.5	20.5	20.6	20.5	20.5
Ca, %	0.44	0.55	0.55	0.55	0.55
P, %	0.55	0.55	0.55	0.55	0.55
STTD P, %	0.46	0.46	0.46	0.46	0.46
Ca:P	0.80	1.00	1.00	1.00	1.00
Na, %	0.35	0.35	0.35	0.35	0.35
Analyzed ABC-4, meq/kg	227	233	240	333	360

[1] Phase 2 diets were fed following phase 1 diets until d 24 post-weaning.

[2] Dairylac 80; International Ingredients Corporation; Fenton, MO.

[3] MEPRO; Prairie Aquatech; Brookings, SD.

[4] Primary Products Ingredients Americas LLC, Decatur, IL

[5] Amasil NA; BASF; Florham Park, NJ.

[6] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.14% STTD P with 1,486 FYT/kg.

Table 3.6 Effects of acidifiers in diets formulated to equal ABC-4 on nursery pig performance and fecal dry matter (DM), Exp. 1[1]

Dietary acidifier:	1[2]	2[3]	3[4]	4[5]	-	-				
ZnO:	-	-	-	-	-	+				
Phase 1 ABC-4, meq/kg:	200	200	200	200	240	327		*P* =		
Phase 2 ABC-4, meq/kg:	250	250	250	250	290	344	SEM	Acidifier[6]	ABC-4[7]	ZnO[8]
Item: BW, kg										
d 0	6.1	6.1	6.1	6.1	6.0	6.0	0.02	0.970	0.795	0.953
d 10	7.3	7.2	7.3	7.4	7.3	7.6	0.14	0.796	0.726	0.254
d 24	11.3	11.2	11.2	11.1	11.1	11.8	0.28	0.934	0.752	0.072
d 38	20.7	20.4	20.2	20.2	20.3	20.6	0.57	0.946	0.907	0.683
Phase 1 (d 0 to 10)										
ADG, g	127	118	121	129	129	151	14.3	0.807	0.699	0.244
ADFI, g	143	135	141	146	151	160	13.3	0.773	0.395	0.518
G:F, g/kg	886	811	856	889	851	918	59.4	0.621	0.883	0.417
Phase 2 (d 10 to 24)										
ADG, g	283	282	281	266	268	296	18.3	0.626	0.495	0.146
ADFI, g	487	468	465	450	482	509	18.4	0.739	0.433	0.305
G:F, g/kg	582	603	603	587	552	582	32.1	0.669	0.009	0.135
Experimental period (d 0 to 24)										
ADG, g	218	213	214	209	210	234	11.6	0.939	0.781	0.131
ADFI, g	344	328	330	323	345	363	13.6	0.928	0.357	0.324
G:F, g/kg	635	643	648	645	607	646	25.0	0.973	0.020	0.052

Common period (d 24 to 38)										
ADG, g	672	657	646	654	662	628	43.2	0.848	0.810	0.107
ADFI, g	938	920	937	929	946	916	52.1	0.852	0.513	0.312
G:F, g/kg	716	715	690	705	699	684	11.1	0.094	0.376	0.199
Overall (d 0 to 38)										
ADG, g	383	376	373	373	376	379	15.5	0.980	0.990	0.880
ADFI, g	559	545	554	547	566	565	24.5	0.903	0.357	0.944
G:F, g/kg	685	689	675	683	664	668	7.0	0.310	0.013	0.652
Fecal DM, %[9]										
d 10	25.4	24.3	25.4	24.4	25.8	27.6	1.13	0.707	0.391	0.218
d 17	24.3	21.6	22.7	22.6	23.0	24.6		0.711	0.848	0.269
d 24	23.2	23.4	21.6	22.2	22.3	23.6		0.403	0.357	0.357

[1] A total of 300 pigs (initial BW of 6.1 ± 0.02 kg) were used in a 38-d nursery trial. A total of 6 dietary treatments were utilized with four low ABC-4 diets utilizing dietary acidifiers and two high ABC-4 diets with or without pharmacological levels on Zn from ZnO. Dietary acidifiers were added to achieve an equal ABC-4 level across the four low ABC-4 diets.

[2] Fumaric acid; Tate & Lyle, Decatur, IL. Included at 0.36% of the diet with an ABC-4 of -10,873 meq/kg.

[3] Activate DA; Novus, St. Charles, MO. Included at 0.87% of the diet with an ABC-4 of -4,487 meq/kg.

[4] KEM-GEST; Kemin, Des Moines, IA. Included at 1.01% of the diet with an ABC-4 of -3,800 meq/kg.

[5] ACID-AID; Alltech, Nicholasville, KY. Included at 0.84% of the diet with an ABC-4 of -4,680 meq/kg.

[6] Compares the four low ABC-4 diets. Excludes the high ABC-4 diets.

[7] Compares the mean of the low ABC-4 diets and the high ABC-4 without ZnO. Excludes the diet with ZnO.

[8] Compares the two high ABC-4 diets. Excludes all low ABC-4 diets.

[9] Treatment × day, $P = 0.885$; Treatment, $P = 0.056$; Day, $P < 0.001$. The P-values represented in the data table show the effect of treatment within day.

Table 3.7 Effects of low ABC-4 formulation strategies on nursery pig performance and fecal dry matter (DM), Exp. 2[1]

Fumaric acid:	+	+	+	-	-				
Low ABC-4 Strategy:	Low Ca[2]	Formic acid[3]	Crystalline lactose[4]	-	-				
ZnO:	-	-	-	-	+				
Phase 1 ABC-4, meq/kg:	200	200	193	300	387		*P* =		
Phase 2 ABC-4, meq/kg:	250	250	262	350	404	SEM	Strategy[5]	ABC-4[6]	ZnO[7]
Item: BW, kg									
d 0	5.9	5.9	5.9	5.9	5.9	0.09	0.175	0.497	0.758
d 8	6.7	6.7	6.6	6.6	6.8	0.07	0.436	0.099	< 0.001
d 24	12.5	12.7	12.4	12.4	13.2	0.29	0.132	0.268	< 0.001
d 38	20.5	21.0	20.3	20.3	21.0	0.30	0.068	0.394	0.073
Phase 1 (d 0 to 8)									
ADG, g	97	100	96	87	117	6.3	0.641	0.055	< 0.001
ADFI, g	120	117	113	114	134	5.7	0.492	0.611	< 0.001
G:F, g/kg	812	848	855	756	867	26.7	0.686	0.006	0.003
Phase 2 (d 8 to 24)									
ADG, g	368	377	361	362	397	15.7	0.138	0.407	< 0.001
ADFI, g	511	511	480	501	542	16.7	0.024	0.973	0.003
G:F, g/kg	722	738	753	724	734	28.5	0.165	0.126	0.345
Experimental period (d 0 to 24)									
ADG, g	277	284	273	270	303	9.7	0.162	0.200	< 0.001
ADFI, g	381	380	358	372	406	11.7	0.031	0.926	0.001

G:F, g/kg	731	750	763	728	748	24.6	0.202	0.022	0.054
Common period (d 24 to 38)									
ADG, g	566	589	565	568	556	16.7	0.094	0.625	0.405
ADFI, g	829	841	816	835	818	13.5	0.170	0.643	0.333
G:F, g/kg	684	702	694	680	681	14.9	0.649	0.062	0.928
Overall (d 0 to 38)									
ADG, g	384	397	381	379	396	7.9	0.079	0.288	0.068
ADFI, g	546	549	526	542	557	10.3	0.048	0.887	0.209
G:F, g/kg	704	722	724	700	711	6.5	0.789	0.005	0.128
Fecal DM, %[8]									
d 8	22.9	21.7	24.3	21.5	22.7	1.70	0.001	0.024	0.150
d 17	24.9	24.7	24.8	23.2	24.1		0.913	0.018	0.288
d 24	24.5	25.1	24.9	23.1	24.2		0.784	0.009	0.179

[1] A total of 725 pigs (initial BW of 5.9 ± 0.09 lb) were used in a 38-d nursery trial across three rooms. A total of 5 dietary treatments were utilized with three low ABC-4 diets utilizing different low ABC-4 formulation strategies. In addition, two high ABC-4 diets were utilized with or without pharmacological levels on Zn from ZnO.

[2] Reducing the Ca:P ratio by 0.20.

[3] Amasil NA; BASF, Florham Park, NJ.

[4] Replacing whey permeate (Dairylac 80; International Ingredients Corporation, St. Charles, MO) with crystalline lactose based on lactose equivalence.

[5] Compares the 3 strategies to achieve a low ABC-4 level. Excludes high ABC-4 diets.

[6] Compares the mean of the low ABC-4 diets and the high ABC-4 diet without ZnO. Excludes the high ABC-4 diet with ZnO.

[7] Compares the two high ABC-4 diets. Excludes all low ABC-4 diets.

[8] Treatment × day, $P = 0.414$; Treatment, $P < 0.001$; Day, $P < 0.001$. The P-values represented in the data table show the effect of treatment within day.

Chapter 4 - Effects of butyrate-based feed ingredient supplementation on weanling pig performance, fecal dry matter, serum chemistry, and intestinal short chain fatty acid concentration

Abstract

Two experiments were conducted to determine the effects of butyrate-based feed additives on weanling pig performance, fecal dry matter (DM), serum chemistry, and intestinal short chain fatty acids. In Exp. 1, 300 pigs, initially 5.5 kg, were used in a 38-d trial with 5 pigs per pen and 10 replications per dietary treatment fed in three phases. Six dietary treatments consisted of a control diet that did not contain any carbadox or pharmacological levels of Zn and Cu, four diets that contained butyrate-based feed ingredients added at 0.1% to the control diet in all phases consisting of encapsulated butyrate, monobutyrin, a mono-tributyrin (MTB) blend, and tributyrin (Eastman; Kingsport, TN). In addition, there was a positive control diet that contained 3,000 mg/kg of Zn, 2,000 mg/kg of Zn, and 250 mg/kg of Cu in phases 1, 2, and 3, respectively, and 55 mg/kg of carbadox (Mecadox; Phibro Animal Health Corp.; Teaneck, NJ) in all phases. Overall (d 0 to 38), pigs fed the positive control had increased ($P < 0.05$) BW, ADG, and d 10 fecal DM compared to all other treatments. Pigs fed MTB had increased ($P < 0.05$) G:F compared to monobutyrin with pigs fed other treatments intermediate. Pigs fed MTB had the greatest ($P \leq 0.046$) d 10 hematocrit and hemoglobin without significant mean separation. Pigs fed MTB had increased cecal butyrate compared to the control with pigs fed other treatments intermediate. In Exp. 2, 2,238 pigs, initially 5.1 kg, were used in a 42-d trial with 26 or 27 pigs per pen and 14 replications per dietary treatment fed in three phases. Six dietary treatments consisted of a control diet, four MTB blends (Eastman; Kingsport, TN), and a commercial feed

additive (AviPlus S; Vetagro Inc., Chicago, IL). The control diet contained 3,000 and 2,000 mg/kg of Zn in phases 1 and 2, respectively, and 55 mg/kg of carbadox in both phases. Each feed additive was added to the control at 0.3% in phases 1 and 2, and 0.1% in phase 3. On d 10, pigs fed the blend of 33% monobutyrin and 66% tributyrin had increased ($P < 0.05$) fecal DM compared to the predominantly monobutyrin blend and commercial additive with pigs fed other treatments intermediate. Pigs fed the blend of 66% monobutyrin and 33% tributyrin and the blend of 33% monobutyrin and 66% tributyrin had decreased ($P < 0.05$) GM-CSF and IFNγ compared to the primarily monobutyrin blend. In summary, MTB can increase cecal butyrate, hematological components, and modulate inflammatory status of weanling pigs.

Key Words: Butyrate, fecal dry matter, inflammatory status, short chain fatty acids, weanling pigs

Introduction

Weanling pigs typically experience reductions in growth performance and increased prevalence of diarrhea. These occurrences can be attributed to intestinal disruptions caused by the sharp transition from sow's milk to a diet containing complex proteins, complex carbohydrates, and antinutritional factors (Heo et al., 2012; Wensley et al., 2021). In addition, pathogenic bacteria such as *Escherichia coli* and *Salmonella* challenge the health status and nutrient utilization of young pigs by diminishing intestinal barrier function and inducing intestinal inflammation (Kim et al., 2022). Historically, the use of growth promoting antibiotics and pharmacological levels of Zn and Cu have been used in weanling pig diets because of their ability to inhibit pathogenic bacteria, enhance gut morphology, and maintain gut integrity (Højberg et al., 2005, Gaskins et al., 2006; Sun et al., 2019). However, dietary use of antibiotics

and Zn and Cu at pharmacological levels has raised concerns surrounding modification of bacterial populations (Bonetti et al., 2021; Monger et al., 2021).

Short chain fatty acids (SCFA) are organic fatty acids with 1 to 6 carbons that are produced within the intestinal lumen via bacterial fermentation. They serve as the main energy source for epithelial cells that line the small and large intestine (Salvi and Cowles, 2021). The three primary SCFA are acetate (C2), propionate (C3), and butyrate (C4). Although butyrate is the least abundant SCFA, it is a major metabolite for epithelial cells and is estimated to provide 60 to 70% of the energy demand for colonocytes (Jacobi and Odle, 2012; Liu et al., 2015). Butyrate glycerides consist of butyric acid attached to a glycerol backbone and can include mono-, di-, and tributyrin additives (Kaur et al., 2016). In the small intestine, butyrate is enzymatically separated from the glycerol backbone for absorption into the upper gastrointestinal tract (Bedford and Gong, 2018). Butyrate in weanling pig diets has been proposed to stimulate intestinal development and improve digestive capabilities (Bedford and Gong, 2018; Tugnoli et al., 2020).

Recent research has shown benefits to butyrate glycerides supplementation as a method to promote healthy gut microbial communities, improve gut morphology, modulate inflammatory cytokines and tight junctions, and ultimately increase growth performance (Chen et al., 2021; Miragoli et al., 2021; Marchiori et al., 2024). Added butyrate glycerides have also been observed to decrease the concentration of pathogenic bacteria such as *E. coli* in the feces and small intestine of weanling pigs (Wang et al., 2019b; Zhang et al., 2020). There is limited research comparing various butyrate glycerides and their efficacy in a commercial environment, which necessitated this research.

Our hypothesis was that butyrate-based feed ingredients will improve gastrointestinal health criteria such as fecal DM, antioxidant capacity, immune responses, and intestinal SCFA concentrations and subsequently increase growth performance. Based on this, we hypothesized that butyrate-based feed ingredient supplementation may serve as a potential alternative to dietary antibiotics and pharmacological levels of Zn and Cu.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Experiment 1 was conducted at the Kansas State Swine Teaching and Research Center in Manhattan, KS. Experiment 2 was conducted at a commercial research site owned and operated by New Horizon Farms, Pipestone, MN. Both sites used two identical nursery rooms that are completely enclosed, environmentally controlled, and mechanically ventilated to maintain a room temperature between 18 and 32°C for weight ranges 5 to 30 kg.

Experiment 1

Animals and treatment structure. A total of 300 pigs (DNA 241 × 600, initially 5.6 ± 0.02 kg) were used in a 38-d trial with 5 pigs per pen and 10 replications (pens) per treatment. Pigs were weaned at approximately 19-d of age and randomly assigned to 1 of 6 dietary treatments based on initial body weight. Dietary treatments were arranged in a one-way treatment structure and fed in 3 phases. Phase 1 was fed from d 0 to 10, phase 2 from d 10 to 24, and phase 3 from d 24 to 38 post-weaning. Dietary treatments consisted of a control, a positive control, and 4 different butyrate-based feed additives added to the control (Table 1). The control

was a standard corn-soybean meal-based diet with no antibiotics or pharmacological levels of Zn or Cu. The control contained 110 mg/kg of Zn and 17 mg/kg of Cu provided by the trace-mineral premix. The positive control diet contained 3,000 and 2,000 mg/kg of Zn from ZnO in phases 1 and 2, respectively and 250 mg/kg of Cu from $CuSO_4$ in phase 3. In addition, the positive control also contained 55 mg/kg of carbadox (Mecadox; Phibro Animal Health Corporation; Teaneck, NJ) across all three dietary phases. The four butyrate-based feed additives were added at 0.1% in the control diet in all three phases and consisted of an encapsulated butyrate, monobutyrin, a mono-tributyrin blend, and tributyrin (Eastman; Kingsport, TN). The mono-tributyrin blend contained 71.5% monobutyrin and 28.5% tributyrin. Diets were formulated to contain 1.35% (phase 1 and 2) and 1.30% standardized ileal digestible (SID) Lys and met or exceeded other amino acid requirements as a ratio to Lys (NRC, 2012). Basal diets for phases 1 and 2 were manufactured at Hubbard Feeds in Beloit, KS. The basal diets were divided into six batches of feed and feed additives were added and mixed at the Kansas State University O.H. Kruse Feed and Technology Innovation Center in Manhattan, KS to form the six experimental diets. Phase 3 diets were manufactured at Hubbard Feeds in Beloit, KS. All diets were offered in meal form. Individual pig weights and feed disappearance were measured on d 10 and weekly afterwards to calculated ADG, ADFI, and G:F.

Fecal samples were collected on d 5, 10, 17, and 24 to determine fecal dry matter (DM) from the same three medium weight pigs per pen. After collection, fecal samples were dried at 55°C in a forced air oven and the ratio of dried to wet fecal weight determined fecal percentage DM.

Blood was collected from the same medium weight pig per pen on d 10 and 38 into one serum blood tube and one K2 EDTA anti-coagulant blood collection tube. Serum blood tubes

were spun down using a centrifuge and serum was submitted for haptoglobin and vitamin E analysis. Haptoglobin was measured using a spectrophotometric evaluation (cobas c501; Roche Diagnostics, Rotkreuz, Switzerland) and vitamin E was analyzed using high-pressure liquid chromatography (Kansas State Veterinary Diagnostic Laboratory in-house method). The K2 EDTA anticoagulant tube was submitted for a complete blood count analysis. All samples were submitted for analysis to the Kansas State Veterinary Diagnostic Laboratory in Manhattan, KS.

After the 38-d study, 6 pigs per treatment group were humanely euthanized via penetrating captive bolt. Approximately 4 mL of digesta was collected from the duodenum, proximal jejunum, ileum, and cecum for analysis of SCFA. The pigs euthanized for digesta were the same pigs used for blood collection on d 10 and 38. Digesta was analyzed using gas chromatography and measured concentration of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaporate, caporate, and heptanoate. Any sample with non-detectable SCFA concentrations was assumed to have a concentration of 0.50 µmol (lower detection limit).

Experiment 2

Animal and treatment structure. A total of 2,238 pigs (PIC 337 × 1050, initially 5.1 ± 0.13 kg) were used in a 42-d study with 26 or 27 pig per pen and 14 replications (pens) per treatment. Pigs were weaned at approximately 21-d of age and randomly assigned to 1 of 6 dietary treatments based on initial body weight and weaning date. Dietary treatments were fed in three phases with phases 1 and 2 fed according to a feed budget of 2.3 and 5.4 kg/pig, respectively. Following phase 2, pigs were fed phase 3 diets until the completion of the study on d 42. The control diet was a standard corn-soybean meal-based diet containing 3,000 and 2,000 mg/kg of Zn from ZnO in phases 1 and 2, respectively (Table 2). The control diet also contained 55 mg/kg of carbadox in phases 1 and 2. The phase 3 control diet did not contain any antibiotics

or pharmacological levels of Zn and Cu. The next four dietary treatments consisted of different mono-tributyrin feed additive blends (Eastman; Kingsport, TN). The first blend primarily consisted of monobutyrin. The second blend was approximately 66% monobutyrin and 33% tributyrin. The third blend was approximately 33% monobutyrin and 66% tributyrin. The fourth blend was a combined mixture of monobutyrin, tributyrin, and C8 and C10 monoglycerides. The final diet used a commercially available feed additive (AviPlus S; Vetagro Inc., Chicago, IL) that is a combination of micro-encapsulated sorbic acids, citric acids, synthetic thymol, and vanillin botanicals. All five feed additives were added at 0.3% in the control diet in phases 1 and 2, and 0.1% of the control diet in phase 3. Diets were formulated to contain 1.35% (phase 1 and 2) and 1.30% SID Lys and met or exceeded other amino acid concentrations as a ratio to Lys (NRC, 2012). Phase 1 diets were manufactured in pellet form at Hubbard Feeds in Mankato, MN. Phase 2 and 3 diets were manufactured in meal form at the New Horizon feed mill in Pipestone, MN.

Pens of pigs were weighed and feed disappearance was calculated weekly to determine ADG, ADFI, and G:F. Under the circumstance where a pig died or needed to be removed from the study due to sickness or injury, the weight of the pig, the pen they originated from, the reason for removal, and date of removal was recorded for analysis. Fecal samples were collected on d 10, 24, and 42 to determine fecal DM from the same three medium weight pigs per pen. Fecal DM analysis followed the same procedure as Exp. 1.

Blood was collected from the same medium weight pig per pen on approximately d 10, 24, and 38. On each day, one serum blood tube and one K2 EDTA anti-coagulant blood collection tube were collected. Serum samples were submitted for haptoglobin, and a cytokine profile and the K2 EDTA anti-coagulant tube was submitted for a complete blood count. The cytokine profile was conducted by Eve Technologies in Calgary, AB Canada using multiplexing

analysis using the Luminex 200 system (Luminex, Austin, TX). Haptoglobin analysis was conducted by the Kansas State University Diagnostic Laboratory in Manhattan, KS. The complete blood count was conducted by the South Dakota State University Animal Research and Diagnostic Laboratory in Brookings, SD.

Statistical analysis

In Exp. 1, data were analyzed as a completely randomized design with pen as the experimental unit. In Exp. 2, data were analyzed as a randomized complete block design with initial body weight and weaning date serving as a blocking factor and pen as the experimental unit. All data were analyzed using the RStudio environment [Version 4.0.2 (2020-06-22), R Core Team, R Foundation for Statistical Computing, Vienna, Austria]. Preplanned pairwise comparisons using the Tukey–Kramer adjustment were used to evaluate differences in treatment means. Fecal dry matter, haptoglobin, vitamin E, complete blood count, and serum cytokines were analyzed using the fixed effects of day, treatment, and the associated interaction accounting for repeated measures over time. Differences between treatments and day (where appropriate) as well as their interaction were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P \leq 0.10$.

Results

Experiment 1

In phase 1 (d 0 to 10), pigs fed the positive control diet had increased ($P < 0.05$) BW compared to pigs fed encapsulated butyrate and the mono-tributyrin blend with those fed other treatments intermediate (Table 3). Pigs fed the positive control had increased ($P < 0.05$) ADG compared to pigs fed the control, encapsulated butyrate, and the mono-tributyrin blend with

those fed other treatments intermediate. Pigs fed the positive control had increased ($P < 0.05$) G:F compared to pigs fed encapsulated butyrate, the mono-tributyrin blend, and tributyrin with those fed other treatments intermediate. In phase 2 (d 10 to 24), pigs fed the positive control had increased ($P < 0.05$) d 24 BW, ADG, and ADFI compared to pigs fed all other treatments. In phase 3 (d 24 to 38), pigs fed the positive control had increased ($P < 0.05$) ADFI compared to pigs fed the mono-tributyrin blend and tributyrin with those fed other treatments intermediate. Pigs fed the mono-tributyrin blend had increased ($P < 0.05$) G:F compared to pigs fed monobutyrin with pigs fed other treatments intermediate. Overall (d 0 to 38), pigs fed the positive control had increased ($P < 0.05$) final BW and ADG compared to pigs fed all other treatments. Pigs fed the positive control had increased ($P < 0.05$) ADFI compared to pigs fed all other treatments except for monobutyrin which was intermediate. Pigs fed the mono-tributyrin blend had increased ($P < 0.05$) G:F compared to pigs fed monobutyrin with all other treatments intermediate.

There was a treatment × day interaction observed ($P = 0.035$) for fecal DM where on d 5, there were no differences in fecal DM between treatments, but on d 10, pigs fed the positive control diet had increased ($P < 0.05$) fecal DM compared to pigs fed all other treatments. On d 17, there were no differences in fecal DM between treatments. On d 24, while a treatment effect was present ($P = 0.003$), no treatment means were significantly different; however, pigs fed the positive control diet and the mono-tributyrin blend had the greatest fecal DM. Averaged across all treatments, fecal DM decreased ($P < 0.05$) from d 5 to 10 and then increased ($P < 0.05$) from d 10 to 17 with no differences ($P > 0.05$) from d 17 to 24.

There was no treatment × day interaction observed for serum haptoglobin concentration (Table 4). On d 38, pigs fed encapsulated butyrate had increased ($P < 0.05$) serum haptoglobin

concentration compared to pigs fed the positive control diet with other treatments intermediate. Serum haptoglobin concentration increased ($P < 0.05$) from d 10 to 38 across all treatments.

There was a treatment × day interaction observed ($P < 0.001$) for serum vitamin E where pigs fed tributyrin had greater ($P < 0.05$) vitamin E concentration on d 10 compared to pigs fed all other treatments. However, there were no differences in serum vitamin E concentration between treatments on d 38 ($P > 0.05$). Serum vitamin E concentration decreased ($P < 0.001$) from d 10 to 38.

For complete blood count analysis, there was a treatment × day interaction ($P = 0.023$) observed for band neutrophil concentrations where there were no differences between treatments on d 10, but pigs fed the mono-tributyrin blend had increased ($P < 0.05$) band neutrophil concentration on d 38 compared to pigs fed all other treatments. For eosinophil concentrations on d 38, while a treatment effect was present ($P = 0.029$) no treatment means were significantly different; however, pigs fed the mono-tributyrin blend and tributyrin had the greatest eosinophil concentrations. For basophil concentrations on d 38, pigs fed the control diet had increased ($P < 0.05$) basophil concentration compared to pigs fed the positive control diet with pigs fed all other treatments intermediate. For hemoglobin and hematocrit concentrations on d 10, while a treatment effect was present ($P \leq 0.046$), no treatment means were significantly different from others; however, pigs fed the mono-tributyrin blend and tributyrin had the greatest hemoglobin and hematocrit concentration.

There were only 5 pigs out of the 36 sampled that had detectable levels of acetate present in the duodenum (Table 5). Pigs fed the mono-tributyrin blend and tributyrin did not have any detectable levels of acetate in the duodenum. In the jejunum, there were no differences between treatments for acetate concentration, although pigs fed the positive control diet were the only

ones that did not have any detectable levels. In the ileum, there were no differences between treatments for acetate or butyrate concentration. For propionate, one pig fed encapsulated butyrate had detectable levels in the ileum. In the cecum, there were no differences between treatments for acetate or propionate concentration. Pigs fed the mono-tributyrin blend had higher cecal ($P < 0.05$) butyrate concentration compared to pigs fed the control with all other treatments intermediate. Pigs fed monobutyrin had higher ($P < 0.05$) valerate concentration compared to pigs fed the positive control with all other treatments intermediate.

Experiment 2

For the duration of the trial, there were no significant differences between treatments for growth performance, removals, and mortalities (Table 6). There was no treatment × day interaction observed for fecal DM; however, pigs fed the blend of 33% monobutyrin and 66% tributyrin had increased ($P < 0.05$) fecal DM compared to pigs fed the primarily monobutyrin blend and the commercial additive on d 10 of the study with pigs fed other treatments intermediate. Fecal DM increased ($P < 0.001$) from d 10 to 42 across all treatments.

There was a treatment × day interaction ($P = 0.041$) observed for blood fibrinogen where pigs fed the primarily monobutyrin blend diets had increased ($P < 0.05$) fibrinogen concentrations compared to pigs fed the commercial additive on d 10 of the study, but no differences between treatments were observed on d 24 or 42 (Table 7). Red blood cell count on d 42 was increased ($P < 0.05$) for pigs fed diets containing the mono-tributyrin blend with C8 and C10 monoglycerides compared to pigs fed the blend of 33% monobutyrin and 66% tributyrin diets with other treatments intermediate. On d 10, pigs fed the control diet had increased ($P < 0.05$) mean corpuscular volume compared to pigs fed diets containing the mono-tributyrin blend with C8 and C10 monoglycerides with other treatments intermediate. Mean corpuscular

hemoglobin concentration on d 42 was increased ($P < 0.05$) for pigs fed the control diet compared to the pigs fed the blend of 66% monobutyrin and 33% tributyrin, the blend of 33% monobutyrin and 66% tributyrin, and the commercial additive diets with other treatments intermediate.

There was no significant treatment × day interactions for any serum cytokines measured. On d 10, pigs fed the blend of primarily monobutyrin and the commercial additive had increased ($P < 0.05$) granulocyte-macrophage colony stimulating factor (GM-CSF) concentration compared to pigs fed the blend of 66% monobutyrin and 33% tributyrin with those fed other treatments intermediate. Also on d 10, pigs fed the blend of primarily monobutyrin had increased ($P < 0.05$) interferon gamma (IFNγ) concentration compared to pigs fed the blend of 66% monobutyrin and 33% tributyrin and the blend of 33% monobutyrin and 66% tributyrin with other treatments intermediate. On d 42, there was a treatment effect ($P = 0.036$) for interleukin 12 (IL-12) concentrations without mean separation with pigs fed the blend of 66% monobutyrin and 33% tributyrin having the highest IL-12 concentration.

Discussion

Butyrate is produced via fermentation of dietary fiber by colonic bacteria. While the main function of butyrate is supplying colonocytes with energy, it also provides other health benefits such as supporting the immune system, reducing inflammation, and preventing enteric disorders (Dang et al., 2023). In addition, butyrate has been observed to mediate protein and gene expression playing a role in development and maintenance of the gastrointestinal tract (Smith et al., 1998). Once butyrate is produced or enters the gastrointestinal tract it is readily metabolized by the gut epithelium. It is estimated that 85 to 90% of butyrate within the colon will be metabolized in the intestinal wall (Knudsen et al., 2003). However, fermentation capabilities to

produce butyrate are limited in newly weaned pigs until approximately 7 to 8 weeks of age (Pluske, 2016; Hunting et al., 2021). Therefore, weanling pigs may benefit from added dietary butyrate to maintain intestinal structure and function.

The majority of research surrounding butyrate-based feed additives in swine diets focuses on either sodium butyrate or tributyrin. The current study focused on butyrate glycerides, specifically mono- and tributyrin, in addition to an encapsulated butyrate used in Exp. 1. Free butyrate supplementation is rarely used because of unpleasant odors and because it is largely absorbed in the upper gastrointestinal tract where it is not able to serve as a colonic energy source (Bedford and Gong, 2018). Therefore, encapsulation techniques or butyrate derivatives are commonly used. Tributyrin consists of three butyric acid molecules that are attached to a glycerol backbone. Tributyrin releases butyric acid through the action of lipase to promote intestinal health and development (Wu and Tsai, 2004). Monobutyrin is a single butyric acid molecule that is positioned on the first carbon atom of the glycerol backbone. Monobutyrin interacts with bacteria in the gastrointestinal tract to control pathogenic proliferation (Wang et al., 2024). Each feed additive has been observed to have beneficial effects on intestinal health and pig performance, but there is limited research comparing the two glycerides and possible synergistic effects from a blend of the two.

In Exp. 1, supplementation of butyrate glycerides was successful at increasing the butyrate concentration within the digesta of the cecum, particularly with the mono-tributyrin blend. However, SCFA concentrations in the small intestine were not affected with or without the inclusion of butyrate-based feed additives. A similar effect was observed in broilers where Hu et al. (2021) observed that increasing tributyrin quadratically increased butyric acid concentration in the cecum of broilers but not earlier in the digestive tract. In contrast, Moquet et

al. (2018) observed broilers fed tributyrin had increased butyrate concentration in the small intestine, with no differences in the cecum. The authors speculated the lack of differences in butyrate found in the large intestine was attributed to the large amount of variation observed. Interestingly, in the current study butyrate-based feed additives influenced cecal valerate concentration indicating differences in the amount of undigested protein entering the large intestine. Research on valerate in pigs is limited, but valerate is typically produced from microbial degradation of amino acids and proteins within the large intestine (Fernandes et al., 2014; Brestenský et al., 2017). Undigested protein that enters the large intestine undergoes microbial fermentation which can increase the occurrence of post-weaning diarrhea (Heo et al., 2009). This agrees with the Exp. 1 fecal DM observations where pigs fed the positive control had the lowest cecal valerate concentration and highest percentage fecal DM. Therefore, pigs fed the positive control diets were likely associated with the least amount of undigested protein entering the large intestine. Experiment 2 also indicated fecal DM can be affected depending on the ratio of monobutyrin to tributyrin fed shortly after weaning. Specifically, the mono-tributyrin blends with a higher proportion of tributyrin led to greater percentage fecal DM. Wang et al. (2019b) also observed increasing levels of tributyrin supplementation linearly improved fecal scores in weanling pigs.

Pigs fed the positive control diet were also associated with decreased haptoglobin at the end of Exp. 1. Haptoglobin is considered a positive acute-phase plasma protein that responds with an increase in concentration based on elevated stress, infection, or inflammation (Murata et al., 2004). Ficagna et al. (2024) also observed that pigs fed diets with feed-grade medications had decreased serum haptoglobin, but similar reductions from feeding butyrate glycerides were not observed in the current study. Interestingly, pigs fed tributyrin in Exp. 1 exhibited increased

serum vitamin E by at least 62% compared to any other treatment. Vitamin E has many roles within the body, but one of the main functions is its natural antioxidant properties (Lúcio et al., 2009). Previous studies have observed butyrate possesses antioxidant capabilities which could explain a sparing effect on vitamin E concentration in pigs fed tributyrin (Canani et al., 2011; Huang et al., 2015).

The cecal valerate concentration, fecal DM, and haptoglobin responses all align with the increased growth rate associated with the positive control in Exp. 1. As expected, the positive control containing carbadox and pharmacological levels of Zn and Cu had improved growth relative to the control. Although pigs fed the positive control diet had the highest growth rate in Exp. 1, pigs fed the mono-tributyrin blend had the greatest feed efficiency suggesting digestibility capabilities were improved from the combination of butyrate glycerides. Marchiori et al. (2024) observed a similar response where pigs fed tributyrin had improved overall feed efficiency compared to a positive control containing antibiotics. The authors suggested the improvements in feed efficiency were from increased pepsin and trypsin release promoting protein digestion. However, this observation may also be partially explained by the increase in hemoglobin and hematocrit concentrations. These hematological components are directly associated with oxygen carrying capacity in blood and nutrient transport capacity (Balcerek et al., 2020). Previous research has also observed improvements in hematological components from feeding butyrate-based feed ingredients in poultry and humans (Witt et al., 2000; Hu et al., 2022). However, these observations in Exp. 1 were not consistent with Exp. 2 based on growth performance, mean corpuscular volume, and corpuscular hemoglobin concentration, which are also indicators of oxygen carrying capacity (Chevalier et al., 2021). These discrepancies could be associated with the fact that butyrate-based feed additives were added alone in Exp. 1 vs. in Exp.

2 they were added to diets also containing dietary feed-grade medications and pharmacological levels of Zn.

Although different mono-tributyrin blends did not influence growth performance or removals and mortalities in Exp. 2, it modulated inflammatory status. Collectively, the blend of 33% monobutyrin and 66% tributyrin had the lowest concentration of the pro-inflammatory cytokines GM-CSF, IFNγ, and IL-12. Previous research has also observed butyrate-based feed additives can inhibit the expression of these pro-inflammatory cytokines (Xu et al., 2016; Chen et al., 2018; Wang et al., 2019a). This observation also aligns with fecal DM being most improved with the blend of 33% monobutyrin and 66% tributyrin indicating less diarrhea presence from less immune pressure. The commercial environment of Exp. 2 allowed the opportunity for differences in inflammatory status to be observed from the various feed additives. The response observed in the pro-inflammatory cytokines for Exp. 2 may also be associated with the response to granulocytes in Exp. 1. For example, GM-CSF is a growth factor that is involved in the recruitment and activity of neutrophils, basophils, and eosinophils (Linden et al., 1999; Laan et al., 2003). Therefore, the ratio of monobutyrin to tributyrin appears to have a large impact on the health status and immune response of weanling pigs.

The results of these experiments indicate that the most effective method to increase growth performance and improve fecal DM of weanling pigs is the use of feed-grade medications and pharmacological levels of Zn and Cu compared to butyrate-based feed ingredients. However, if pharmacological levels of Zn and Cu are ultimately banned or limited for use in swine, a mono-tributyrin blend provided beneficial effects in feed efficiency, cecal butyrate concentration, and hematological concentrations. Results of Exp. 2 also indicate that pigs fed a blend of 33% monobutyrin and 66% tributyrin provided the most benefit for

inflammatory status and fecal DM compared to other mono-tributyrin blends in commercial environment.

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Table 4.1 Experimental diet composition (as-fed basis), Exp. 1

	Phase 1[1]		Phase 2[2]		Phase 3[3]	
Item	Control[4]	Positive Control	Control[4]	Positive Control	Control[4]	Positive Control
Ingredients, %						
Corn	43.90	43.90	46.05	46.05	65.10	64.35
Soybean meal (46.5% CP)	21.95	21.95	30.75	30.75	31.30	31.30
Dried distillers grains with solubles	5.00	5.00	7.50	7.50	---	---
Whey powder	10.00	10.00	---	---	---	---
Whey permeate	10.00	10.00	10.00	10.00	---	---
Microbial enhanced soybean meal[5]	3.50	3.50	---	---	---	---
Choice white grease	1.00	1.00	1.00	1.00	---	---
Limestone	0.60	0.20	0.80	0.45	0.70	0.35
Monocalcium phosphate (21% P)	1.15	1.15	1.00	1.00	0.95	0.95
Salt	0.30	0.30	0.50	0.50	0.60	0.60
L-Lys HCl	0.55	0.55	0.50	0.50	0.45	0.45
DL-Met	0.23	0.23	0.21	0.21	0.17	0.17
L-Thr	0.22	0.22	0.22	0.22	0.20	0.20
L-Trp	0.04	0.04	0.03	0.03	0.03	0.03
L-Val	0.15	0.15	0.12	0.12	0.10	0.10
Vitamin premix with phytase[6]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[7]	0.15	0.15	0.15	0.15	0.15	0.15

Zinc oxide	---	0.41	---	0.27	---	---
Copper sulfate	---	---	---	---	---	0.10
Mecadox[8]	---	1.00	---	1.00	---	1.00
Sand	1.01	---	0.92	---	---	---
Total	100	100	100	100	100	100
SID amino acids, %						
Lys	1.35	1.35	1.35	1.35	1.30	1.30
Ile:Lys	55	55	57	57	57	57
Leu:Lys	112	112	118	118	119	119
Met:Lys	37	37	37	37	35	35
Met and Cys:Lys	55	55	57	57	57	57
Thr:Lys	63	63	64	64	64	64
Trp:Lys	19.1	19.1	19.2	19.2	19.1	19.1
Val:Lys	70	70	70	70	70	70
His:Lys	33	33	36	36	38	38
Total Lys, %	1.49	1.49	1.51	1.51	1.44	1.44
NE NRC, kcal/kg	2,502	2,502	2,449	2,449	2,427	2,427
SID Lys:NE, g/Mcal	5.39	5.39	5.51	5.51	5.36	5.36
CP, %	20.3	20.3	21.7	21.7	21.1	21.1
Ca, %	0.69	0.69	0.71	0.71	0.63	0.63
P, %	0.66	0.66	0.63	0.63	0.60	0.60

STTD P, %	0.58	0.58	0.51	0.51	0.46	0.46

[1] Phase 1 diets were fed from approximately 5.4 to 6.4 kg.

[2] Phase 2 diets were fed from approximately 6.4 to 10.9 kg.

[3] Phase 3 diets were fed from approximately 10.9 to 18.1 kg.

[4] Encapsulated butyrate, monobutyrin, tributyrin, and a mono-tributyrin blend (Eastman Chemical, Kingsport, TN) were all added at 0.1% of the control diet. The mono-tributyrin blend was approximately 71.5% monobutyrin and 28.5% tributyrin.

[5] ME-PRO, Prairie Aquatech, Brookings, SD.

[6] Ronozyme HiPhos 2700 (DSM, Parsippany, NJ) provided an estimated release of 0.13% STTD P with 1,252 FTU/kg for all three phases. Provided per kg of diet: 4,134 IU vitamin A; 1,653 IU vitamin D; 44 IU vitamin E; 3.3 mg vitamin K; 0.03 mg vitamin B12; 50 mg niacin; 28 mg pantothenic acid; 8 mg riboflavin.

[7] Provided per kg of diet: 110 mg zinc; 110 mg iron; 33 mg manganese; 17 mg copper; 0.3 mg iodine; 0.3 mg selenium.

[8] Mecadox (Phibro Animal Health Corp., Teaneck, NJ) provided 55 mg/kg of carbadox in the diet.

Table 4.2 Experimental diet composition (as-fed basis), Exp. 2[1]

Item	Phase 1 control[2]	Phase 2 control[3]	Phase 3 control
Ingredients, %			
Corn	38.51	56.35	56.21
Soybean meal (46.5% CP)	22.00	20.28	24.77
Whey powder	25.00	10.00	---
Dried distillers grains with solubles	---	---	15.00
Enzymatically treated soybean meal[4]	7.00	7.00	---
Soybean oil	3.00	---	---
Corn oil	---	1.00	---
Limestone	0.60	0.70	1.30
Monocalcium phosphate (21% P)	1.15	1.15	0.45
Salt	0.35	0.55	0.60
L-Lys HCl	0.35	---	---
Liquid Lys (55%)	---	0.75	0.85
DL-Met	0.22	0.24	0.16
L-Thr	0.15	---	---
Thr-Pro[5]	---	0.30	0.28
L-Trp	0.02	0.05	0.05
L-Val	0.08	0.14	0.09
Vitamin premix[6]	0.05	---	---
Trace mineral premix[7]	0.08	---	---
Vitamin-trace mineral premix[8]	---	0.20	0.20
Selenium premix	0.05	---	---
Phytase[9]	0.02	0.05	0.05
Zinc oxide	0.40	0.27	---
Mecadox[10]	1.00	1.00	---
Total	100	100	100
SID amino acids, %			
Lys	1.35	1.35	1.30

Ile:Lys	62	55	56
Leu:Lys	116	111	128
Met:Lys	36	38	35
Met and Cys:Lys	58	58	58
Thr:Lys	64	65	65
Trp:Lys	19.4	19.3	19.1
Val:Lys	70	69	69
His:Lys	35	34	37
Total Lys, %	1.48	1.49	1.46
NE NRC, kcal/kg	2,579	2,478	2,418
SID Lys:NE, g/Mcal	5.24	5.45	5.38
CP, %	21.1	20.4	21.6
Ca, %	0.85	0.79	0.68
P, %	0.73	0.66	0.55
STTD P, %	0.62	0.55	0.42

[1] Each feed additive was added to the control diet at 0.30% in phases 1 and 2, and 0.1% in phase 3. Feed additives included: a primarily monobutyrin blend, a blend of 66% monobutyrin and 33% tributyrin, a blend of 33% monobutyrin and 66% tributyrin, a mono-tributyrin blend with C8 and C10 monoglycerides (MCFA; Eastman Chemical, Kingsport, TN), and a commercially available feed additive (AviPlus S; Vetagro Inc., Chicago, IL).

[2] Phase 1 diets were provided with a feed budget of 2.3 kg/pig.

[3] Phase 2 diets were provided with a feed budget of 5.4 kg/pig.

[4] HP 300, Hamlet Protein, Findlay, OH.

[5] CJ America, Los Angeles, CA.

[6] Provided per kg of diet: 12,128 IU vitamin A; 2,205 IU vitamin D; 66 IU vitamin E; 7 mg vitamin K; 0.06 mg vitamin B12; 50 mg niacin; 45 mg pantothenic acid; 9 mg riboflavin; 0.2 mg biotin; 1 mg folic acid; 3 mg pyridoxine.

[7] Provided per kg of diet: 120 mg zinc; 101 mg iron; 30 mg manganese; 10 mg copper; 0.5 mg iodine.

[8] Provided per kg of diet: 8,820 IU vitamin A; 1,442 IU vitamin D; 88 IU vitamin E; 4 mg vitamin K; 0.04 mg vitamin B12; 60 mg niacin; 29 mg pantothenic acid; 8 mg riboflavin; 0.2 mg

biotin; 1 mg pyridoxine; 105 mg zinc; 120 mg iron; 40 mg manganese; 18 mg copper; 0.3 mg iodine; 0.3 mg selenium; 198 µg chromium.

[9] Phase 1: Quantum Blue 5G (ABVista, Plantation, FL) which provided an estimated release of 0.12% STTD P with 752 FTU/kg. Phase 2 and 3: Optiphos Plus 2500 G (Huvepharma Inc.; Peachtree City, Georgia) which provided an estimated release of 0.13% STTD P with 1,250 FTU/kg.

[10] Mecadox (Phibro Animal Health Corp., Teaneck, NJ) provided 55 mg/kg of carbadox in the diet.

Table 4.3 Effects of butyrate-based feed additives on nursery pig growth performance and fecal dry matter, Exp. 1[1,2]

Protein source:	Control[3]	Positive Control[4]	Encapsulated Butyrate[5]	Monobutyrin[5]	Mono-Tributyrin[5,6]	Tributyrin[5]	SEM	*P* =
BW, kg								
d 0	5.5	5.6	5.5	5.6	5.6	5.5	0.02	0.922
d 10	6.1[ab]	6.4[a]	6.1[b]	6.2[ab]	6.1[b]	6.1[ab]	0.13	0.013
d 24	10.7[b]	12.5[a]	10.5[b]	11.3[b]	10.8[b]	10.7[b]	0.26	< 0.001
d 38	17.9[b]	19.8[a]	17.6[b]	17.9[b]	17.4[b]	17.5[b]	0.41	< 0.001
Day 0 to 10 (Phase 1)								
ADG, g	57[b]	90[a]	53[b]	64[ab]	53[b]	63[ab]	12.8	0.006
ADFI, g	116	136	129	133	118	127	10.8	0.283
G:F, g/kg	490[ab]	665[a]	414[b]	474[ab]	431[b]	439[b]	74.4	0.007
Day 10 to 24 (Phase 2)								
ADG, g	324[b]	435[a]	320[b]	361[b]	337[b]	324[b]	14.2	< 0.001
ADFI, g	385[b]	512[a]	399[b]	435[b]	396[b]	384[b]	19.3	< 0.001
G:F, g/kg	841	850	801	837	854	850	27.4	0.439
Day 24 to 38 (Phase 3)								
ADG, g	514	525	504	473	477	488	19.1	0.289
ADFI, g	749[ab]	803[a]	736[ab]	752[ab]	683[b]	709[b]	21.1	0.003
G:F, g/kg	686[ab]	653[ab]	685[ab]	629[b]	696[a]	686[ab]	13.8	0.008
Day 0 to 38 (Overall)								
ADG, g	321[b]	377[a]	318[b]	323[b]	314[b]	316[b]	10.4	< 0.001

ADFI, g	444[b]	520[a]	452[b]	470[ab]	429[b]	436[b]	14.6	< 0.001
G:F, g/kg	723[ab]	726[ab]	703[ab]	687[b]	732[a]	723[ab]	9.5	0.012
Fecal DM, %[8]								
d 5	19.8	23.7	22.4	21.4	20.8	22.2	1.16	0.243
d 10	15.9[b]	23.6[a]	16.3[b]	15.3[b]	16.7[b]	13.7[b]		< 0.001
d 17	18.6	21.8	18.3	18.9	19.1	18.2		0.249
d 24	19.8	22.5	18.2	17.9	22.5	18.0		0.003

[1] A total of 300 pigs (initial BW of 5.5 ± 0.02 kg) were used in a 38-d nursery trial with 5 pigs per pen and 10 pens per treatment spread across two rooms. Pigs were weaned at approximately 19-d of age and allotted to treatment in a completely randomized design.

[2] Pens of pigs were fed diets in 3 phases. Pigs were fed phase 1 diets from 0 to 10 d after weaning, phase 2 diets from d 10 to 24, and phase 3 diets from d 24 to 38.

[3] The control consisted of a standard corn-soybean meal-based diet with no added antibiotics, pharmacological levels of Zn or Cu, and butyrate-based feed additives

[4] The positive control contained 3,000 and 2,000 mg/kg of Zn from ZnO in phases 1 and 2, respectively. In phase 3, the positive control contained 250 mg/kg of Cu from $CuSO_4$. In all three phases, the positive control also contained 55 mg/kg of carbadox from Mecadox across all three phases.

[5] All butyrate-based feed additives (Eastman Chemical, Kingsport, TN) were added at 0.1% of the control diet.

[6] The mono-tributyrin blend contained 71.5% monobutyrin and 28.5% tributyrin.

[7] F/G was calculated by taking the inverse of G:F. Therefore, no SEM is reported and *P*-values are consistent with G:F.

[8] Treatment × day, $P = 0.035$; Treatment, $P < 0.001$; Day, $P < 0.001$. The *P*-values represented in the data table show the effect of treatment within day. The SEM is representative of all collection periods.

Table 4.4 Effects of butyrate-based feed additives on nursery pig haptoglobin, vitamin E, and a complete blood count, Exp. 1[1]

Protein source:	Control[2]	Positive Control[3]	Encapsulated Butyrate[4]	Monobutyrin[4]	Mono-Tributyrin[4,5]	Tributyrin[4]	SEM	*P* =
Haptoglobin, mg/dL[6]								
d 10	69	35	49	81	67	66	17.1	0.334
d 38	100[ab]	48[b]	130[a]	101[ab]	117[a]	101[ab]		0.007
Vitamin E, mg/kg[7]								
d 10	0.54[b]	0.45[b]	0.64[b]	0.50[b]	0.68[b]	1.09[a]	0.085	< 0.001
d 38	0.49	0.35	0.40	0.36	0.54	0.37		0.359
Complete blood count								
Band neutrophil concentration, K/uL[8]								
d 10	0.03	0.00	0.00	0.00	0.00	0.00	0.011	0.223
d 38	0.00[b]	0.00[b]	0.00[b]	0.00[b]	0.05[a]	0.00[b]		0.008
Eosinophil concentration, K/uL[9]								
d 10	0.20	0.20	0.14	0.08	0.11	0.16	0.126	0.975
d 38	0.49	0.32	0.43	0.30	0.70	0.76		0.029
Basophil concentration, K/uL								
d 10	0.01	0.01	0.05	0.00	0.01	0.05	0.027	0.519
d 38	0.11[a]	0.00[b]	0.07[ab]	0.02[ab]	0.03[ab]	0.04[ab]		0.049
Hematocrit, %[9]								
d 10	42.5	42.1	43.7	43.1	46.5	45.3	1.18	0.046
d 38	39.3	38.2	39.8	39.7	39.5	37.3		0.621

Hemoglobin, g/dL[9]								
d 10	12.8	12.7	13.0	12.9	13.7	13.6	0.29	0.044
d 38	11.3	11.2	11.2	11.5	11.4	10.8		0.535

[1] On d 10 and 38 of the trial, blood was collected from one pig per pen (10 pigs per treatment and 60 pigs total) and analyzed for haptoglobin, vitamin E, and a complete blood count. All samples were submitted to the Kansas State Veterinary Diagnostic Laboratory (Manhattan, KS). The same pig was bled on d 10 and 38. On d 10 of blood collection a total of 6 blood tubes clotted and could not be analyzed for complete blood count (1 positive control, 1 encapsulated butyrate, 1 monobutyrin, 2 mono-tributyrin blend, and 1 tributyrin). On d 38 of blood collection a total of 4 blood tubes clotted and could not be analyzed for complete blood count (1 control, 2 mono-tributyrin blend, and 1 tributyrin).

[2] The control consisted of a standard corn-soybean meal-based diet with no added antibiotics, pharmacological levels of Zn or Cu, and butyrate-based feed additives

[3] The positive control contained 3,000 and 2,000 mg/kg of Zn from ZnO in phases 1 and 2, respectively. In phase 3, the positive control contained 250 mg/kg of Cu from $CuSO_4$. In all three phases, the positive control also contained 55 mg/kg of carbadox from Mecadox across all three phases.

[4] All butyrate-based feed additives (Eastman Chemical, Kingsport, TN) were added at 0.1% of the control diet.

[5] The mono-tributyrin blend contained 71.5% monobutyrin and 28.5% tributyrin.

[6] Treatment × day, $P = 0.272$; Treatment, $P = 0.009$; Day, $P < 0.001$. The *P*-values represented in the data table show the effect of treatment within day.

[7] Treatment × day, $P < 0.001$; Treatment, $P = 0.001$; Day, $P < 0.001$. The *P*-values represented in the data table show the effect of treatment within day.

[8] Treatment × day, $P < 0.05$.

[9] Day, $P < 0.05$.

Table 4.5 Effects of butyrate-based feed additives on intestinal short chain fatty acid composition, Exp. 1[1,2]

Item:	Control[3]	Positive Control[4]	Encapsulated Butyrate[5]	Monobutyrin[5]	Mono-Tributyrin[5,6]	Tributyrin[5]	SEM	*P* =
Acetate, µmol/L								
Duodenum	3.99 (1/6)	1.04 (1/6)	0.91 (2/6)	0.77 (1/6)	<0.50 (0/6)	<0.50 (0/6)	1.453	0.520
Jejunum	6.35 (4/6)	<0.50 (0/6)	1.28 (3/6)	1.62 (4/6)	2.84 (5/6)	3.45 (6/6)	2.159	0.469
Ileum	21.1 (6/6)	18.4 (6/6)	11.4 (6/6)	54.2 (6/6)	15.7 (6/6)	31.45 (6/6)	17.595	0.566
Cecum	62.17 (6/6)	62.95 (6/6)	66.55 (6/6)	63.32 (6/6)	66.60 (6/6)	63.20 (6/6)	2.600	0.734
Propionate, µmol/L								
Ileum	<0.50 (0/6)	<0.50 (0/6)	1.02 (1/6)	<0.50 (0/6)	<0.50 (0/6)	<0.50 (0/6)	0.210	0.435
Cecum	28.71 (6/6)	30.15 (6/6)	31.04 (6/6)	29.43 (6/6)	30.05 (6/6)	30.36 (6/6)	0.877	0.533
Butyrate, µmol/L								
Ileum	2.71 (5/6)	2.43 (5/6)	0.97 (2/6)	10.24 (4/6)	1.54 (3/6)	3.59 (4/6)	3.483	0.465
Cecum	14.19^{b} (6/6)	15.12^{ab} (6/6)	17.02^{ab} (6/6)	18.70^{ab} (6/6)	20.40^{a} (6/6)	16.01^{ab} (6/6)	1.335	0.026
Valerate, µmol/L								
Cecum	2.64^{bc} (6/6)	1.21^{c} (4/6)	3.26^{ab} (6/6)	4.31^{a} (6/6)	3.47^{ab} (6/6)	2.80^{b} (6/6)	0.350	<0.001

[1] On d 38 of the trial, 6 medium weight pigs from each treatment were humanely euthanized via penetrating captive bolt. Samples of digesta were taken from the duodenum, proximal jejunum, ileum, and cecum for analysis of short chain fatty acids. Pigs euthanized for digesta

collection were the same pigs bled on d 10 and 38 of the trial. Digesta was analyzed using gas chromatography and measured concentrations of acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, isocaporate, caporate, and heptanoate.

[2] Any sample with non-detectable levels of SCFA was assumed to have a concentration of 0.50 µmol (lower detection limit). Short chain fatty acids not reported in the table indicates all samples were below the lower detection limit. Number in parentheses indicate the number of samples with detectable levels.

[3] The control consisted of a standard corn-soybean meal-based diet with no added antibiotics, pharmacological levels of Zn or Cu, and butyrate-based feed additives

[4] The positive control contained 3,000 and 2,000 mg/kg of Zn from ZnO in phases 1 and 2, respectively. In phase 3, the positive control contained 250 mg/kg of Cu from $CuSO_4$. In all three phases, the positive control also contained 55 mg/kg of carbadox from Mecadox across all three phases.

[5] All butyrate-based feed additives (Eastman Chemical, Kingsport, TN) were added at 0.1% of the control diet.

[6] The mono-tributyrin blend contained 71.5% monobutyrin and 28.5% tributyrin.

Table 4.6 Effects of butyrate-based ingredients on nursery pig growth performance, removals and mortality, and fecal dry matter, Exp. 2[1]

		Mono-tributyrin blends						
Item:	Control[2]	Primarily monobutyrin[3]	66% monobutyrin 33% tributyrin[3]	33% monobutyrin 66% tributyrin[3]	Mono-tributyrin w/ MCFA[4]	Commercial additive[5]	SEM	*P* =
BW, kg								
d 0	5.1	5.1	5.1	5.1	5.1	5.1	0.13	0.999
d 7	5.6	5.6	5.6	5.7	5.6	5.6	0.13	0.988
d 21	9.9	10.0	9.9	10.0	10.1	10.0	0.20	0.593
d 42	18.0	18.2	17.9	18.5	18.3	18.1	0.35	0.352
d 42 per pig placed	15.7	15.6	14.7	16.1	15.4	15.6	0.48	0.217
Day 0 to 7[6]								
ADG, g	76	74	66	78	70	71	6.8	0.803
ADFI, g	167	167	158	169	162	166	5.5	0.631
G:F, g/kg	452	440	415	462	418	428	36.7	0.897
Day 7 to 21[7]								
ADG, g	290	300	288	301	303	299	8.2	0.463
ADFI, g	378	389	385	386	399	393	9.0	0.481
G:F, g/kg	766	774	748	778	761	760	11.3	0.379
Day 21 to 42[8]								
ADG, g	376	378	373	396	381	380	10.2	0.351
ADFI, g	564	561	559	591	580	564	12.4	0.222

G:F, g/kg	666	675	667	670	655	674	8.9	0.338
Day 0 to 42 (Overall)								
ADG, g	292	296	286	305	296	295	6.6	0.234
ADFI, g	429	431	424	445	441	433	8.1	0.365
G:F, g/kg	681	687	673	687	672	682	6.4	0.182
Removals and mortality								
Removals, %[9]	8.8	10.2	12.6	9.7	11.3	9.4	1.71	0.630
Mortality, %[10]	2.9	2.7	3.8	2.7	3.2	2.1	0.96	0.887
Total mortality[11]	4.3	5.4	7.0	4.8	6.7	5.1	1.28	0.600
Total rem and mort, %	11.8	12.9	16.4	12.3	14.5	11.5	1.88	0.408
Fecal DM (%)[12]								
d 10	19.1[ab]	17.8[b]	20.5[ab]	21.4[a]	18.2[ab]	17.8[b]	0.86	0.008
d 24	21.0	21.7	21.7	22.9	22.5	22.5		0.604
d 42	24.9	24.6	25.8	25.3	26.3	25.8		0.718

[1] A total of 2,238 pigs (initial BW of 5.1 ± 0.13 kg) were used in a 42-d nursery trial with 26 or 27 pigs per pen and 14 pens per treatment spread across two rooms. Pigs received a feed budget of 2.3 and 5.4 kg/pig for phases 1 and 2, respectively. Following phase 2, pigs were fed phase 3 diets until the completion of the study.

[2] The control consisted of a standard corn-soybean meal-based diet with 3,000 and 2,000 mg/kg of Zn from ZnO in phase 1 and 2, respectively. The control diet also contained 55 mg/kg carbadox (Mecadox; Phibro Animal Health Corp., Teaneck, NJ) in phase 1 and 2.

[3] Eastman Chemical, Kingsport, TN. All products were added at 0.3% of the control diet in phase 1 and 2, and 0.1% of the control diet in phase 3.

[4] A mono-tributyrin blend with C8 and C10 monoglycerides (MCFA; Eastman Chemical, Kingsport, TN) added at 0.3% of the control diet in phase 1 and 2, and 0.1% of the control diet in phase 3.

[5] AviPlus S; Vetagro, Chicago, IL. Added at 0.3% of the control diet in phase 1 and 2, and 0.1% of the control diet in phase 3.

[6] Approximate feeding period of phase 1 diets.

[7] Approximate feeding period of phase 2 diets.

[8] Approximate feeding period of phase 3 diets.

[9] Percentage of pigs that were removed into a hospital pen.

[10] Percentage of pigs that died/euthanized in original pen.

[11] Percentage of pigs that died/euthanized in original pen or hospital pen after being removed.

[12] Treatment × day, $P = 0.269$; Treatment, $P = 0.078$; Day, $P < 0.001$. The *P*-values represented in the data table show the effect of treatment within day.

Table 4.7 Effects of butyrate-based ingredients on nursery pig complete blood count and serum cytokines Exp. 2[1]

		Mono-tributyrin blends						
Item:	Control[2]	Primarily monobutyrin[3]	66% monobutyrin 33% tributyrin[3]	33% monobutyrin 66% tributyrin[3]	Mono-tributyrin w/ MCFA[4]	Commercial additive[5]	SEM	*P* =
Complete blood count[6]								
Red blood cell count, M/uL[7]								
d 10	6.14	6.38	6.40	6.21	6.66	6.41	0.258	0.238
d 24	5.60	5.46	5.41	5.39	5.55	5.33		0.817
d 42	5.87^{ab}	5.86^{ab}	5.84^{ab}	5.46^{b}	6.21^{a}	5.77^{ab}		0.064
Mean corpuscular volume, fL[7]								
d 10	63.3^{a}	62.4^{ab}	61.8^{ab}	62.4^{ab}	57.7^{b}	61.4^{ab}	1.95	0.022
d 24	60.4	59.7	59.5	60.1	59.3	60.8		0.940
d 42	60.1	59.4	59.7	59.1	58.4	59.5		0.957
Mean corpuscular hemoglobin concentration, g/dL								
d 10	30.7	31.5	30.5	31.6	31.8	31.7	1.12	0.644
d 24	30.7	30.9	30.8	30.9	30.6	30.5		0.997
d 42	33.6^{a}	31.0^{ab}	30.6^{b}	30.7^{b}	30.8^{ab}	30.4^{b}		0.022
Fibrinogen, mg/dL[7,8]								
d 10	292^{ab}	409^{a}	215^{ab}	226^{ab}	233^{ab}	171^{b}	104.6	0.020
d 24	371	308	417	282	300	400		0.272
d 42	282	292	331	325	355	250		0.796
Serum cytokine, fluorescence intensity[9]								

GM-CSF[8]								
d 10	82.3[abc]	124.1[a]	27.0[c]	40.4[b]	43.0[b]	107.5[ab]	19.64	<0.001
d 24	19.5	26.1	23.4	19.7	26.1	24.8		1.000
d 42	19.7	21.3	22.4	25.4	22.1	18.9		1.000
IFNγ[8]								
d 10	283.5[ab]	621.1[a]	189.2[b]	205.2[b]	298.9[ab]	289.0[ab]	94.95	0.020
d 24	61.8	54.8	48.5	114.0	160.9	50.0		0.947
d 42	45.7	45.3	31.3	47.7	56.5	29.1		1.000
IL-12[7,8]								
d 10	1,007	1,128	1,188	958	976	974	149.0	0.834
d 24	1,480	1,081	1,520	1,292	1,147	1,278		0.224
d 42	1,956	1,720	2,092	1,514	1,567	1,588		0.030

[1] On d 10, 24, and 42 of the trial, blood was collected from one pig per pen (14 pigs per treatment and 84 pigs total) and analyzed for complete blood count to the South Dakota State Animal Disease Research and Diagnostic Laboratory (Brookings, SD) and analyzed for Porcine Cytokine/Chemkine 13-Plex Discovery Assay to Eve Technologies (Calgary, AB Canada). The same pig was bled on d 10, 24, and 42.

[2] The control consisted of a standard corn-soybean meal-based diet with 3,000 and 2,000 mg/kg of Zn from ZnO in phase 1 and 2, respectively. The control diet also contained 55 mg/kg carbadox (Mecadox; Phibro Animal Health Corp., Teaneck, NJ) in phase 1 and 2.

[3] Eastman Chemical, Kingsport, TN. All products were added at 0.3% of the control diet in phase 1 and 2, and 0.1% of the control diet in phase 3.

[4] A mono-tributyrin blend with C8 and C10 monoglycerides (MCFA; Eastman Chemical, Kingsport, TN) added at 0.3% of the control diet in phase 1 and 2, and 0.1% of the control diet in phase 3.

[5] AviPlus S; Vetagro, Chicago, IL. Added at 0.3% of the control diet in phase 1 and 2, and 0.1% of the control diet in phase 3.

[6] On d 10 of blood collection a total of 9 blood tubes clotted and could not be analyzed for complete blood count (1 control, 3 blend of primarily monobutyrin, 1 blend of 66% monobutyrin and 33% tributyrin, 2 blend of 33% monobutyrin and 66% tributyrin, and 2 mono-tributyrin blend with MCFA). On d 24 of blood collection a total of 9 blood tubes clotted and could not be analyzed for complete blood count (2 blend of primarily monobutyrin, 2 blend of 66% monobutyrin and 33% tributyrin, 4 blend of 33% monobutyrin and 66% tributyrin, and 1 mono-tributyrin blend with MCFA). On d 42 of blood collection a total of 17 blood tubes clotted and could not be analyzed for complete blood count (3 control, 2 blend of primarily monobutyrin, 1 blend of 66% monobutyrin and 33% tributyrin, 2 blend of 33% monobutyrin and 66% tributyrin, 3 mono-tributyrin blend with MCFA, and 6 commercial additive).

[7] Day, $P < 0.05$. The *P*-values represented in the data table show the effect of treatment within day.

[8] Treatment × Day, $P < 0.05$.

[9] The median reporter fluorescence intensity value of the bead population for a specific analyte in arbitrary fluorescence intensity units.

Chapter 5 - Nutritional guide to feeding wheat and wheat co-products to swine: a review

Abstract

Inclusion of wheat grain can offer feeding opportunities in swine diets because of its high starch, crude protein (CP), amino acid (AA), and phosphorus (P) content. High concentrations of starch within wheat grain makes it a good energy source for swine diets. Mean energy content of wheat was 4,900 and 3,785 kcal/kg DM for digestible energy and metabolizable energy, respectively. Crude protein concentration can vary based on the class of wheat which include hard red winter, hard red spring, soft red winter, hard white, soft white, and durum. The average CP of all wheat data collected in this review was 12.6% with a range of 8.5 to 17.6%. The AA concentration of wheat increases with increasing CP with the mean Lys content of 0.38% with a standardized ileal digestibility (SID) of 76.8%. As CP of wheat increases, the SID of AA in wheat also increases. Mean P of wheat was 0.27% and median P was 0.30%. Off-quality wheat is often associated with sprouts, low-test weight, or mycotoxin-contamination. Sprouted and low-test weight wheat are physical abnormalities associated with decreased starch within wheat kernel that leads to reductions in energy. The assumed energy value of wheat grain may need to be reduced by up to 10% when the proportion of sprouted to non-sprouted wheat is up to 40% whereas above 40%, wheat's energy may need to be reduced by 15 to 20%. Low-test weight wheat appears to not influence pig performance unless it falls below 644 kg/m^3 and then energy value should be decreased by 5% compared to normal wheat. Deoxynivalenol (DON) contamination is most common with wheat grain. When content is above the guidance level of 1 mg/kg of DON in the complete diet, each 1 mg/kg increase in a DON contaminated wheat-based diet will result in a 11 and 6% reduction in ADG and ADFI for nursery pigs, and a 2.7 and 2.6%

reduction in ADG and ADFI, in finishing pigs, respectively. Wheat co-products are produced from the flour milling industry. Wheat co-products include wheat bran middlings, millrun, shorts, and red dog. Wheat co-products can be used in swine diets, but application may change because of differences in the final diet energy concentration due to changes in the starch and fiber levels of each wheat co-product. However, feeding wheat co-products are being evaluated to improve digestive health. Overall, wheat and wheat co-products can be fed in all stages of production if energy and other nutrient characteristics are considered.

Key Words: Off-quality wheat, swine, wheat, wheat by-products, wheat co-products

Introduction

Because of their starch content (45-65%), cereals grains serve as the main energy source in swine diets. Wheat is a major cereal grain that is produced throughout the world for both human and livestock consumption. Worldwide wheat production has continued to steadily increase and totaled approximately 781 million tonnes in 2023, which ranks second among cereal grains (USDA, 2023). Wheat can offer many useful properties in swine diets because of its nutrient profile, digestibility, enzymatic activity, and pelleting characteristics (Stein et al., 2010). However, depending on the class of wheat, the nutrient content may vary.

The quality of wheat can be affected due to adverse weather conditions, delayed harvest, or improper storage resulting in “off-quality” wheat. The three most common occurrences are sprouted, low-test weight, and mycotoxin-contaminated wheat. Sprouted and low-test weight wheat are physical abnormalities associated with reduced energy content and possibly other nutrient alterations if severely damaged (Murray and Huber, 1968; Hines and Pollman 1982). Mycotoxins are produced by molds growing on wheat grain which can lead to reduced

performance in pigs depending on the type of mycotoxin and the concentration. Although feeding off-quality wheat may result in diminished performance, it may be priced at a discount which presents an economic opportunity in swine diets (Marston and DeRouchey, 2004). However, it is important to understand handling and feeding strategies of off-quality wheat.

Wheat that is processed in flour milling intended for the human food industry produces various co-products. Wheat co-products include wheat bran, middlings, millrun, shorts, and red dog (AAFCO, 2023). Depending on the area of the world, availability of wheat co-products is variable. Wheat middlings are most common in the United States, wheat millrun is common in Canada, and wheat bran is more common in Europe. Wheat co-product classification is dependent on the combination and concentrations of different fractions within the co-products (Huang et al., 2012). Therefore, wheat co-products vary in their nutrient profile, AA profile, and digestibility. Clear characterization of these co-products is warranted for consistency across the industry and application into swine diets.

The first objective of this review was to provide an understanding of nutritional properties associated with wheat grain and where there may be advantages as an energy source in swine diets. The second objective of this review was to provide an understanding on the effects of feeding off-quality wheat on pig performance and how it can be effectively incorporated into swine diets. The last objective of this review was to clearly characterize wheat co-products and provide insight into potential applications in swine diets to reduce cost.

Procedures

The current review utilized a literature search to compile previously published peer-reviewed journal articles to summarize nutrient, digestibility, and processing characteristics of wheat and wheat co-products. The search was performed via the Kansas State University

Libraries under the CAB Direct and PubMed database, in addition to Google Scholar. Search terms including (WHEAT OR WHEAT CO-PRODUCTS OR WHEAT BY-PRODUCTS OR WHEAT BRAN OR WHEAT MIDDLINGS OR WHEAT MILLRUN OR WHEAT SHORTS OR WHEAT RED DOG) AND (PIGS OR SWINE) AND the respective search item of interest (cereal grain, protein, amino acid, phosphorus, energy, standardized ileal digestibility, growth performance, nursery, finishing, sows, carcass, particle size, pelleting, pellet durability, pelleting aid, pellet quality, off-quality, sprouted, test-weight, mycotoxin, deoxynivalenol, zearalenone, ochratoxin, ergot alkaloids, fumonisin, aflatoxin, fiber, starch, gastrointestinal, microbiome, and intestinal). Articles were not restricted based on region but were restricted to primarily English language. Once relative articles were identified, they were filed and categorized according to topic for further evaluation.

Wheat grain nutrient data was collected from 38 published peer-reviewed journal articles totaling 55 wheat samples. Wheat standardized ileal digestible (SID) data was obtained from 11 published peer-reviewed journal articles totaling 30 wheat samples. Wheat energy values were collected from 10 published peer-reviewed journal articles totaling 28 wheat samples. The time frame for data selection was restricted from 2013 to 2024 to provide an accurate representation of wheat based on recent literature. Additionally, there are previous publications summarizing wheat nutrient characteristics before 2013 (NRC, 2012; Rosenfelder et al., 2013). For off-quality wheat, no year restriction was enforced because of little available research. A total of 11 published peer-reviewed journal articles were collected with a total of 22 DON contaminated wheat-based diets for a regression analysis on nursery and finishing pig growth performance.

Wheat co-product nutrient data was collected from 42 published peer-reviewed journal articles totaling 121 wheat co-product samples. Mean nutrient values were summarized from 28

wheat bran, 48 wheat middlings, 5 wheat millrun, 21 wheat shorts, and 19 wheat red dog samples. Data on SID of crude protein (CP) and amino acids (AA) of wheat co-products was collected from 17 published peer-reviewed journal articles consisting of 5 wheat bran, 27 wheat middlings, 7 wheat shorts, and 6 wheat red dog samples. Using data from 10 journal articles, mean energy values were developed from 12 wheat bran, 21 wheat middlings, 9 wheat shorts, and 10 wheat red dog samples. The time frame for data selection was restricted from 2008 to 2024 to provide an accurate representation of wheat based on recent literature. In contrast to the wheat section, the wheat co-product literature search was extended to the last 15 years because of a relevant journal article from Nortey et al. (2008) comparing various wheat co-products.

A main objective of this review was to characterize co-product nutrient differences between wheat bran, wheat middlings, wheat millrun, wheat shorts and wheat red dog. As publications were reviewed, the wheat co-products were initially classified based on the co-product terminology utilized in the journal article. The classification was either confirmed or re-classified based on Association of American Feed Control Officials (AAFCO; 2023) definitions and nutrient outliers. A total of 11 wheat co-product samples had to be re-classified. The basis of the reclassification to a different co-product class was due to the reported values of CF, starch or insoluble fiber.

Feeding Wheat Grain to Swine

Classes of wheat

Discussion on classes of wheat is primarily based on U.S. classification, although many other countries and regions utilize the same or similar classifications for wheat. Wheat is classified based on hardness (hard or soft), color (red or white), and growing season (winter or spring; Table 1). Hardness is an indication of protein concentration within the endosperm of the

wheat kernel, specifically the protein to starch ratio and the physical force it takes to fracture the kernel (Saleem et al., 2022). Assuming 88% dry matter (DM), hard wheat is typically classified as having greater than 10.5% protein and soft wheat is generally lower according to U.S. Wheat Associates (2022). However, depending on various factors such as growing region, weather, cultivar, soil nitrogen, harvest time, etc., hard and soft classes of wheat can fall outside of the protein classification. The ideal protein content of wheat is based on its end use for the food industry. Typically, flour intended for bread utilizes high protein wheat (hard) whereas flour for cakes or pastries preferentially utilizes lower protein (soft) wheat. Wheat is also classified based on kernel color. Red wheat has a reddish hue, whereas white wheat has a sandy beige color. At harvest, red wheat is typically more resistant to sprouting in wet regions, whereas white wheat is better suited for dry conditions (Grafenauer et al., 2020). Lastly, wheat varieties can be planted and harvested at different times of the year. As the name implies, spring wheat is usually planted in the spring and harvested in the fall, whereas winter wheat is planted in the fall, becomes dormant during the winter, and is harvested the following summer (Hall and Nleya, 2019). Winter wheat requires a period of vernalization to initiate the reproductive stage following initial growth (Shourbalal et al., 2019). Winter wheat requires temperature to be less than 8°C for vernalization in the winter followed by flowering in the spring (Amasino, 2004). The Canadian Grain Commission (2023) further classifies wheat based on growing location within the country. Canadian wheat can either be classified as eastern, northern, prairie, or western. Historically, hard red winter and soft red winter are most common for livestock feed, while hard red spring may also be fed depending on the year (USDA, 2023). Canadian western red spring wheat is the most common wheat fed in Canada and represents approximately 62% of total wheat production (Agriculture and Agri-Food Canada, 2024).

Hard red winter wheat is the most common wheat class in the U.S. representing approximately 34% of all wheat production (Figure 1; USDA, 2023). It is grown in a variety of areas across the country including the great plains (Kansas, Oklahoma, Texas, and North Dakota), the pacific northwest (Montana and Washington), and California (Figure 2 and 3). It is utilized either alone or in combination with other classes of wheat for general all-purpose flour. Hard red winter wheat provides consistency for the milling process because of its availability and nutritional properties (U.S. Wheat Associates, 2022).

Hard red spring wheat represents approximately 24% of all U.S. wheat production (USDA, 2023) which is mostly grown in the north central region of the U.S. (Montana and North Dakota). Hard red spring is known for its high protein content ranging from 12 to 15% protein making it an appealing wheat class for hard breads such as bagels and pizza crust. It also provides improved flour yield relative to other wheat classes because of its dense endosperm. Hard red spring wheat is further divided into subclasses consisting of dark northern spring, northern spring, and red spring. These subclasses are an indication of the percentage of dark, hard, vitreous kernels present (U.S. Wheat Associates, 2022).

For the eastern third of the U.S., soft red winter wheat is the most common class of wheat representing 25% of all wheat production (USDA, 2023). This class of wheat is primarily grown in Illinois, Indiana, Arkansas, North Carolina, and many other eastern states. The soft endosperm of soft red winter wheat results in a lower protein content of 8.5 to 10.5%. However, soft red winter wheat is often blended with hard red classes of wheat to improve the texture and appearance of the products it is used to produce (U.S. Wheat Associates, 2022).

Soft white wheat represents approximately 13% of all wheat production (USDA, 2023). It is mostly found in the pacific northwest (Washinton, Idaho, and Oregon) and consists of 3

subclasses. Subclasses are an indication of the amount of club wheat present and include soft white, white club, and western white. Club wheat is a subspecies that is blended with conventional soft white wheat to form subclasses (Johnson et al., 2008). An appealing quality of soft white wheat is a low moisture content which is typically less than 10%. Its high DM allows for optimization of flour extraction, reduced particle size, and appearance of its end products (U.S. Wheat Associates, 2023). However, similar to soft red winter wheat, protein is lower and ranges from 8.5 to 10.5%.

The least common class of wheat in the U.S. is hard white wheat only representing 1% of all wheat production (USDA, 2023). Hard white wheat has both winter and spring varieties which are mostly grown in western Kansas and Nebraska. Hard white wheat can have a high protein content containing up to 14% depending on the growing season. Hard white wheat is associated with decreased polyphenol oxidase compared to other wheat classes (Park et al., 1997). Polyphenol oxidase is an enzyme that is responsible for undesirable browning of wheat products (Demeke and Morris, 2002).

The last class of wheat is durum, the hardest of all wheat varieties. Durum's protein ranges from 12 to 15% making it an appealing wheat variety for human consumption. It is an excellent class for pasta production. Durum production represents only 3.3% of all U.S. wheat production (USDA, 2023). Durum is mostly grown in North Dakota, but some varieties can also be found in Arizona and California often referred to as desert durum. Subclasses are an indication of the percentage of hard, vitreous, amber colored kernels which include hard amber durum, amber durum, or durum if it fails to meet the other two subclass criteria (U.S. Wheat Associates, 2023).

Standards for wheat

Wheat is graded on a scale of 1 to 5 with 1 representing high quality wheat and 5 representing poor quality wheat (Table 2; USDA, 2014). Factors affecting the U.S. grade of wheat in a single lot include the class of wheat, contrasting classes of wheat, damaged kernels, dockage, foreign material, other grains, and sieve size. The minimum test weight affects the U.S. grade depending on the class of wheat. For example, hard red winter must meet a test weight of 60.0 lb/bu (772 kg/m^3) to qualify for U.S. grade of 1, whereas hard red spring wheat only requires a test weight of 58.0 lb/bu (747 kg/m^3) for the same grade. A greater percentage of a contrasting class of wheat present will also result in a poorer U.S. grade. For example, hard red winter wheat and soft white wheat are contrasting classes. Therefore, if there is 2% hard red winter wheat within a soft white wheat purchase, the U.S. grade will depreciate from 1 to 2. Similarly, presence of damaged kernels, which can include defects, heat damage, shrunken, and broken kernels, will negatively impact the U.S. grade. Foreign material present will also result in a poorer grade. Foreign material is anything other than the wheat kernels including the presence of other grains. Rescreening or recleaning wheat to remove substances other than wheat can be done to improve the U.S. grade. In addition, near-infrared spectroscopy (NIRS) has emerged as an affordable and reliable method to sort wheat grain and improve the grade (Johnson, 2020). The final factor that affects the U.S. grade is its ability to fit through a 0.064 × 0.375-inch (0.163 × 0.953 cm) oblong-hole sieve. Ultimately, the grade wheat receives will influence its end use whether it is exported, enters the milling process, or is used for livestock.

Energy

Wheat is considered to be a high starch, low fiber cereal grain making it a good energy source for swine diets (McGhee and Stein, 2020). Digestible carbohydrates include

monosaccharides, disaccharides, and polysaccharides such as starch. Starch in wheat is comprised of approximately 20% amylose and 80% amylopectin (Kiem et al., 2014). Non-digestible carbohydrates include oligosaccharides, resistant starch, and non-starch polysaccharides (fiber). On average, wheat is approximately 10.9% total dietary fiber (TDF), most of which is insoluble dietary fiber. Wheat does contain β-glucans and arabinoxylans, which are soluble fibers that have been shown to have beneficial effects on acetate and lactate production within digestive tract of swine (Cervantes-Pahm et al., 2014; Bai et al., 2021).

Mean gross energy (GE), digestible energy (DE), and metabolizable energy (ME) values of wheat were 4,419, 3,393, and 3,831 kcal/kg of DM, respectively. The DE of wheat ranged from 3,456 to 4,126 kcal/kg DM and ME ranged from 3,353 to 3,975 kcal/kg DM. Previous literature suggested wheat contains 91 to 97% of the energy of corn for pigs (Stein et al., 2010). However, results based on recent data suggest wheat's energy is approximately 99 and 98% of the energy of corn for DE and ME, respectively. Similar to protein, energy can be influenced by many factors associated with wheat. Rosenfelder et al. (2013) summarized 15 studies and observed that winter wheat had greater DE, but lower ME compared to spring wheat. The author's suggested these differences may have been associated with increased variation due to methodology, wheat class, and growing conditions. In the current review, there appears to be no correlation between protein (hardness) and DE or ME in wheat. A regression analysis revealed CP was not significantly related ($P > 0.10$) to the DE or ME of the 28 samples of wheat collected from the 10 published peer-reviewed journal articles.

There were no publications found that determined net energy (NE) from the current review's search criteria and restrictions imposed. However, NE can still be calculated from publications that report DE, EE, starch, CP, and ADF values by utilizing Eq. 1-8 from NRC

(2012). The average NE from 16 wheat samples across 5 journal publications was calculated to be 2,786 kcal/kg DM which agrees with previously published wheat NE values. The NRC (2012) reports an NE of 2,788 and 3,004 kcal/kg DM for hard red and soft red wheat, respectively. The CVB (2016) reports wheat NE at 2,888 kcal/kg DM and the INRA (2021) reports NE at 2,900 kcal/kg DM for soft wheat.

Crude protein and amino acids

The CP of wheat samples collected from literature ranged from 8.5 to 17.6% on an as-fed basis (Table 3). Wheat classifications varied among publications with the majority not indicating the wheat class, origin, or region utilized for the study. It is often difficult to classify wheat as hard or soft based on only CP. U.S. Wheat Associates (2022) classifies soft and hard wheat as below or above 10.5% CP, respectively, whereas the NRC (2012) utilizes 11% CP as the separation of classification. In addition, often there is overlap with CP content and wheat classes. For example, Rosenfelder (2015) reported that 7 out of 8 soft wheat samples had greater than 11% CP. In addition, Bloxham et al. (2018) utilized hard red wheat, but one sample only contained 10.2% CP. Factors such as weather, geographic location, and growing season can also influence protein content. Winter wheat is associated with higher yields than spring wheat, which is negatively correlated with protein concentration (Posner, 2000). Therefore, spring wheat classes will generally have higher protein than winter wheat classes. However, varying climate conditions can influence these observations. Many factors can influence the nutrient concentrations of wheat; therefore, nutrient analysis should be performed regularly.

The average CP content across all summarized wheat data was 12.6%. As CP increased within wheat samples, so did AA content. Therefore, a simple linear regression model was developed to estimate the AA concentration of a sample of wheat based on CP (Table 4). All AA

regression coefficients were significant ($P < 0.001$) except for Tyr. All coefficients for the explanatory variable (CP) were positive, indicating an increase in CP will result in an increase in each AA. The coefficient for CP on Lys is 0.019, meaning a 1% increase in CP will result in a 0.019% increase in Lys (Figure 4). Indicated by the R-square value, 33.1% of the variation in Lys concentration is explained by wheat's CP content. This is also the lowest coefficient of determination of all AA. For other indispensable AA, a change in CP will have the greatest unit change in Leu (0.051) and the least change in Trp (0.010). For dispensable AA, a change in CP of wheat will result in the greatest unit change in Glu (0.306) and the least change in Cys (0.026). The variation that is most explained by CP was Ile (72.4%) and Glu (75.9%) for indispensable and dispensable AA, respectively. The variation that is least explained by CP was Cys (43.2%) for dispensable AA. These models can be utilized to predict the approximate AA profile of wheat based on percent CP. However, the models will only provide an estimate on AA content based on CP and should be used with caution.

The mean total starch was 57.7% across all wheat samples from collected literature. As total starch of a wheat sample increased, CP decreased. Starch is a main determinant of wheat protein content. If starch granules do not fill up, structural protein content is indirectly increased (Lalman and Highfill, 2002), both having a major effect on grain energy value. Shang et al. (2020) observed that soft wheat varieties (lower CP) had a larger proportion of total starch compared to hard wheat varieties across twelve wheat samples. For other nutrients such as DM, ether extract (EE), TDF, acid-detergent fiber (ADF), and neutral detergent fiber (NDF), mean values from the current review were similar to previously reported values (Stein et al., 2010; NRC, 2012; Rosenfelder et al., 2013).

Wheat comparisons to other common cereal grains were accomplished by utilizing published journal articles with direct comparisons of wheat to either corn (Table 6), barley (Table 7), or sorghum (Table 8). Overall, wheat is a good source of CP and AA compared to other common cereal grains.

Standardized ileal digestibility of crude protein and amino acids

Generally, mean SID for CP and AA in wheat are similar to previously reported percentages (Stein et al., 2010; NRC 2012). The mean SID of CP and Lys for all wheat samples was 87.2 and 76.8%, respectively. Lysine has the lowest SID of all AA which is consistent with previous literature. Among other cereal grains, wheat is higher in SID of CP and all indispensable AA compared to barley and sorghum. The SID of CP and AA in wheat is similar to corn, however, it has higher SID for Trp. Feed grade Trp is an expensive ingredient compared to other feed grade AA for swine. Therefore, less supplemental Trp is needed when feeding a wheat-based diet compared to a corn-based diet because of both AA content and digestibility. The order of first 5 limiting AA based on SID AA within wheat from collective literature is Trp, Met, Thr, Lys, and His.

In general, as CP of wheat increased the SID of AA in wheat increased as well. Therefore, multiple regression models were developed to predict the content of SID AA within wheat in relation to the CP content (Table 5). These regression equations can be utilized to determine the approximate SID of each AA in a sample of wheat based on its CP concentration. As previously discussed, SID AA within wheat prediction models only provide an estimate based on CP and should be used with caution. Each CP regression coefficient was significant ($P < 0.001$) except for Pro and Tyr. To predict the relationship between CP and the content of SID Lys, a coefficient of 0.026 was found, meaning a 1% change in CP will result in a 0.026%

change in SID Lys of wheat (Figure 5). The SID Lys had the poorest relationship with CP (lowest coefficient of determination) of all AA with significant *P* values (36.2%). Interestingly, it appears the regression equation may overestimate the SID Lys in wheat for samples that are under 12.5% CP and may underestimate the SID Lys in wheat for samples over 12.5% CP. Reasoning for this observation is unclear, but may be associated with the class of wheat, weather during growth, geographic location, and other external factors. For other indispensable AA, an increase in CP will have the greatest unit change on the SID Leu (0.074) and the least change on the SID Met (0.012). Leucine is also the indispensable AA that has the most variation in SID that is explained by CP (75.8%). For dispensable AA, an increase in CP will have the greatest unit change on the SID Glu (0.299) and the least change on the SID Ser (0.029). Glutamine is also the dispensable AA whose variation in SID is most explained by the CP (79.5%). The dispensable AA with the lowest coefficient of determination is Ala (50.5%).

Phosphorus

A major benefit to feeding wheat in diets is its concentration of P compared to corn and other cereal grains. Mean P was 0.27% summarized from this review, which is lower than previously reported values. Stein et al. (2010) reported average wheat P was 0.34% with a range of 0.23 to 0.38%. In addition, the NRC (2012) reported mean P for hard red and soft red wheat was 0.39 and 0.30%, respectively. It is unclear why mean P was lower in the current review but may be a consequence of less soil P from growing environmental concerns leading to less uptake by the plant. However, it is important to note the large standard deviation, with P ranging from 0.07 to 0.45% across all summarized literature. There were five wheat samples with P below 0.16% which resulted in a lower mean P than median P. The median P was 0.30% for all wheat samples. Removing the 5 low P wheat samples increased mean P to match median P of 0.30%.

Although this is lower than previously reported, wheat is still higher in P than other cereal grains such as corn and sorghum.

Wheat contains intrinsic phytase that improves P digestibility in swine. In other cereal grains, a percentage of P is stored in the form of phytate-bound P which is largely unavailable to pigs because the amount of endogenous phytase in the small intestine is negligible (Eeckhout and DePaepe, 1994). Intrinsic phytase within wheat allows for increased release of phytate-bound P to improve P digestibility in pigs and reduce P supplementation in the diet (Poulsen et al., 2010). Brinch-Pedersen et al. (2014) reported the phytase activity of wheat was 1,637 FTU/kg whereas other cereal grains such as corn, rice and sorghum were 190 FTU/kg or less. McGhee and Stein (2019) reported standardized total tract digestibility (STTD) of P was 36.6% in wheat-based diets compared to 24.9% in corn-based diets. When 1,000 FTU/kg of microbial phytase was added to the diets this resulted in an STTD of P at 57.6 and 62.5% for wheat and corn-based diets, respectively. The magnitude of P release is dependent on the cereal grain and amount of added phytase to the diets (Wu et al., 2004). Supplemental dietary P, often in the form of monocalcium or dicalcium phosphate, can be a costly component in swine diets. Therefore, high P and intrinsic phytase will allow for less P supplementation which can provide an economic advantage.

Effect of wheat in swine diets

Historically, research has suggested that wheat can be fed as the main cereal source in swine diets without affecting performance (Gill et al., 1966; Jensen et al., 1969). However, as genetics of both wheat and swine change to meet the goals of each industry, the response to wheat-based swine diets may change as well. In addition, different stages of production may elicit different responses when comparing wheat to other cereal grains.

Nursery pigs

Newly weaned pigs are particularly sensitive to ingredient changes and diet palatability (Wensley et al., 2021). Previous data suggests that wheat-based diets do not affect nursery pig performance when compared to corn-based diets (Rodriguez and Young, 1981). Mejicanos et al. (2017) and Bloxham et al. (2018) observed wheat-based diets did not affect nursery pig feed intake compared to corn-based diets. Both authors observed nursery pigs fed wheat-based diets had on average 6% improved feed efficiency compared to corn-based diets. In both studies, dietary treatments were balanced for energy and SID Lys; however, diet analysis indicated that the wheat-based diets had higher concentrations of GE and EE for the Mejicanos et al. (2017) and Bloxham et al. (2018) studies, respectively. Therefore, the improvements in feed efficiency associated with wheat-based diets may have been due to greater energy density of the diet. It is also possible better nutrient digestibility within wheat and the presence of gut promoting fibers could improve nutrient utilization (Bloxham et al. 2018; Bai et al., 2021). Gut promoting fibers in wheat may be a potential area of opportunity that is discussed more in depth in the wheat co-products portion of the review. Bloxham et al. (2018) also observed that ADG was highest when nursery pigs were fed a cereal blend of 60% corn and 40% wheat rather than a single cereal grain. Fouhse et al. (2016) and Zhou et al. (2016) observed nursery pigs fed barley-based diets resulted in improved ADG and feed efficiency compared to wheat-based diets. However, GE in barley diets from Zhou et al. (2016) was approximately 95 kcal/kg greater than wheat-based diets. Diets fed by Fouhse et al. (2016) were formulated to be isocaloric, but chemical analysis was not reported to confirm the formulation. It does appear differences in performance observed in various studies may be due to varying energy densities of the diet rather than a change in cereal grain.

Although there is little research comparing different classes of wheat, Jha et al. (2011) observed no differences in nursery pig growth performance between pigs fed white spring, red spring, hard red spring, durum, hard red winter, and hard white spring wheat of western Canada. Based on these results, the class of wheat should not affect nursery pig performance if nutritional differences among classes are considered.

Finishing pigs

Feeding wheat in finishing diets can provide certain formulation benefits as previously described, but it is also important to understand how wheat-based diets may influence performance and carcass characteristics. Similar to nursery pigs, Han et al. (2005) observed finishing pigs fed wheat-based diets had improved feed efficiency compared to corn-based diets, when beef tallow was added to the wheat-based diets to balance energy. Carr et al. (2005) observed pigs fed wheat-based diets had decreased ADFI, but no difference in feed efficiency than those fed corn-based diets. Chemical analysis revealed the wheat-based diet was 65 kcal/kg lower in ME than the corn-based diets, but still did not significantly influence ADG or feed efficiency. McConnell et al. (1975) observed growth performance between wheat-based diets and corn-based diets was comparable. For carcass characteristics, McConnell et al. (1975) and Carr et al. (2005) observed no differences when comparing pigs fed wheat-based diets to corn and barley-based diets. In contrast, although Han et al. (2005) did not observe any differences in ADG, pigs fed wheat-based diets had lighter slaughter and carcass weights with all other carcass characteristics unaffected compared to pigs fed corn-based diets. In addition, Wang et al. (2023) observed that although increasing levels of wheat in finishing pig diets did not affect growth performance, it significantly enhanced lightness, redness, and yellowness in the longissimus dorsi muscle. For fat color, Han et al. (2005) observed backfat lightness, redness, and yellowness

was not affected when feeding finishing pigs either a corn or wheat-based diet. Although research shows some variable results, most response criteria in the literature were similar to feeding wheat compared to other cereal grains. Therefore, wheat is a suitable cereal grain for finishing pig performance while the effects on carcass characteristics remains variable and should be considered when implementing a change in cereal grains.

In addition to growth performance and carcass characteristics, wheat-based diets have also been observed to reduce fecal N excretion compared to corn, barley, and sorghum-based diets (Leek et al., 2007; Pan and An, 2020). However, Leek et al. (2007) observed urinary N excretion was greater and total N retention was decreased in pigs fed wheat-based diets compared to corn and barley. If N excretion into the environment is a concern, feeding wheat-based diets can increase N excretion mainly driven by urinary N losses. However, enzymes can be implemented into diets to mitigate this issue.

Research has also shown the class of wheat can affect performance in finishing pigs, in contrast to early findings in nursery pigs. Pigs fed hard red winter wheat had increased ADG and ADFI compared to pigs fed soft red winter wheat and no differences in carcass characteristics (De Jong et al., 2016). The authors speculated that soft red winter wheat may have been lower in energy than expected.

Sows

For sow and litter performance, maximizing sow lactation feed intake is important for optimal milk production to support litter growth. Therefore, it is important to understand how wheat affects ADFI and subsequent performance compared to other cereal grains. Park et al. (2010) observed feeding wheat-based diets led to reduced ADFI, poorer sow body condition, reduced reproductive performance, increased wean-to-estrus interval, and poorer litter

performance than corn-based diets even though diets were balanced for energy. The authors speculated that nutrient digestibility in corn was greater than wheat which resulted in the observations. Although corn and wheat-based diets were formulated to be isocaloric, the corn-based diets had 0.41% greater average fat content compared to wheat-based diets potentially leading to greater energy density associated with corn-based diets. Stein et al. (1999) observed wheat-based diet's apparent total tract digestibility of CP was 16.2% units greater than corn-based diets for both gestating and lactating sows. The authors also observed sows fed wheat-based diets had 0.7 and 1.1 kg greater ADFI during lactation compared to corn and barley-based diets, respectively. Bryant et al. (1985) and Nyachoti et al. (2006) observed similar lactation performance in sows fed either wheat or corn-based diets. However, Bryant et al. (1985) observed sows fed wheat-based diets had decreased total born, live born, and litter birth weight compared to sows fed corn-based diets. Diets were formulated to be isocaloric and isonitrogenous, but wheat-based diets did have 2.2% greater CP and 36 kcal/kg lower GE. Although there is little research available on wheat-based diets in gestation, the greater fiber content associated with wheat may help to increase satiety compared to other cereal grains such as corn and sorghum. Overall, it appears wheat can be utilized effectively in sow diets if energy density is not reduced. To our knowledge there are no studies comparing different classes of wheat in sow diets. The only study to report the class of wheat utilized was Bryant et al. (1985) where they utilized soft winter wheat.

Although there are some inconsistencies in the literature, wheat-based diets appear to be comparable to other cereal grains across all phases of production. Differences observed in literature may be due to different energy densities of the diets rather than a change in cereal grain. Mycotoxin contamination not reported by authors could also explain inconsistencies in the

literature in relation to pig performance which is discussed later in the current review. Based on current literature, the class of wheat has been shown to have an impact in finishing diets, but not nursery pigs. However, further research is warranted in this area.

Processing and pelleting

Determining the optimal particle size from grinding is important for ideal performance in pigs. DeJong et al. (2016) observed a linear improvement in feed efficiency when wheat particle size was reduced from 728 to 326 µm in finishing pigs. In contrast, Bao et al. (2016) observed no differences in feed efficiency with reducing wheat particle size from 670 to 330 µm in growing pigs. The authors did observe a quadratic effect with ADG initially increasing from 670 to 450 µm and then decreasing from 430 to 330 µm. Finer particle sizes are thought to lead to improvements in pig performance because of increased surface area and nutrient digestibility. Grinding wheat too fine has been theorized to cause flour accumulation which may lead to increased stomach keratinization and ulceration. However, Mavromichalis et al. (2000) suggested factors causing stress to finishing pigs is need for ulceration and should not be blamed simply on grinding wheat too fine. Finely ground wheat does increases dust accumulation and requires more electrical power to reach a target particle size. Stein et al. (2010) recommended wheat particle size should be no less than 500 µm for pigs to avoid these potential issues.

Utilizing either a hammer mill or roller mill for wheat particle size reduction can influence the response in pigs. Acosta et al. (2020) observed nutrient digestibility for finishing pigs was best when wheat was ground to 500 µm utilizing a hammer mill. However, when utilizing a roller mill the most optimal particle size for finishing pigs was 700 µm for nutrient digestibility. In growing pigs, the authors observed the optimal particle size was 500 µm regardless of particle size reduction method. Traditionally, roller mills are recommended for

reducing the particle size of wheat because of improved uniformity and fewer fines (Goodband et al., 1995). Choct et al. (2004) observed that diets with wheat ground through a hammer mill significantly improved feed efficiency and numerically improved ADG compared to pigs fed wheat-based diets ground through a roller mill. However, the roller mill diets had a larger average particle size than the hammer mill diets which could have resulted in the feed efficiency response. Regardless of the method to reduce particle size of wheat, grinding to small particle sizes will lead to more electrical consumption and lower throughput (Bao et al., 2016; De Jong et al., 2016). It is important to determine the optimal balance for performance, stomach integrity and feed milling efficiency within a production system for the most economical outcome.

In addition to particle size and grinding method, bulk density should also be a concern especially when utilizing wheat co-products. As wheat co-product inclusion increases, bulk density of the diet decreases because of added fiber. The change in bulk density will be more substantial with high fiber wheat co-products such as wheat bran and middlings which is discussed in the wheat co-products portion of the review. A low bulk density diet can be associated with increased gut fill, limiting pigs' ability to consume enough feed to meet their energy demand. In addition, low bulk density diets can result in poor feed flowability potentially limiting feed accessibility and causing out of feed events. Bulk density of wheat middlings has been suggested as an indicator of assessing wheat middlings quality (Cromwell et al., 2000). Light bulk density wheat middlings are generally associated with more bran material whereas heavier bulk density wheat middlings are associated with more residual endosperm (Cromwell et al., 1992; Salyer et al., 2012).

A strategy to increase bulk density is pelleting swine diets. Pelleted diets will also improve palatability, reduce dust, reduce feed wastage, improve handling and transportation, and

increase nutrient utilization (Lancheros et al., 2020). To achieve these benefits from pelleting, it is important to have high quality pellets for swine. Wheat-based diets are often associated with increased pellet durability because of its gluten and hemicellulose content that are able to bind particles together compared to other cereal grains such as corn (Behnke, 2019). Stevens (1987) and Abdollahi et al. (2010) observed wheat-based diets improved pellet durability index (PDI) by 6.3 and 2.2%, respectively compared to corn-based diets. In contrast, Amerah et al. (2008) observed corn-based diets had increased PDI compared to wheat-based broiler diets. However, all treatments had a PDI of at least 91.5% indicating all had good quality pellets. Even if wheat is not fed as the main cereal grain for a swine ration, it can be used as a pelleting aid. Behnke (1990) and Moradi et al. (2018) observed PDI progressively increased by an average of 1.4, 5.7, and 8.38% when incorporating wheat from 0, 5, 10, and 20%, respectively in corn or sorghum-based diets. Research also suggests the class of wheat can also influence pellet durability. De Jong et al. (2016) and Carré et al. (2005) observed hard wheat classes lead to increased PDI compared to softer classes.

Wheat co-products that contain proportions of the endosperm are also an option to improve pellet durability of swine diets. The endosperm is where gluten-forming proteins are found which are responsible for improving pellet quality (Blancher et al., 2005). Therefore, wheat middlings, millrun, shorts, and red dog would be expected to improve pellet quality, whereas wheat bran would not. Wecker et al. (2018) observed 7.4, 11.6, and 14.4% improvements in PDI when incorporating 2.5, 5, and 10% of a pasta co-product mainly comprised of wheat flour into corn-based diets. Starch gelatinization of cooked wheat co-products improves intra-particle bonds on the surface of the pellet, but over cooking can damage starch particles and diminish pellet quality (Behnke, 2001). Wecker et al. (2018) also observed

10% inclusion of the pasta co-product resulted in significantly increased PDI compared to a commercial pellet binder. Similarly, Saleh et al. (2021) observed 5% inclusion of wheat middlings improved PDI by 2.7% in a corn-based diet. Based on current research, 10% wheat or wheat co-products, with the exception of wheat bran, can be used to increase pellet quality. Pellet quality will continue to improve with higher wheat inclusion, but improvements may be diminishing.

Overall, wheat can provide opportunities in swine diets because of its high concentration and digestibility of nutrients. Although wheat may have lower energy compared to some other cereal grains such as corn, wheat-based diets are high in CP, AA, and P compared to other cereal grains. All aspects will factor into the total diet cost to be able to make decisions on formulation strategies. Furthermore, pelleted swine diets give additional value to wheat compared to meal diets largely because of reduced fines and less feed wastage.

Feeding Off-Quality Wheat

Sprouted wheat

Sprouted grains are seeds that have begun to germinate and are harvested before they grow into a plant (Benincasa et al., 2019). Wet weather conditions can lead to delayed harvest which can cause small grains to sprout after they mature (Thomason et al., 2019). Wheat is particularly sensitive to sprouting, specifically soft classes of wheat that are grown in damp regions with excess rain, fog, and humidity (Nagelkirk, 2017). Sprouted wheat is typically not suitable for the milling industry and is consequently sold at a discount and used in livestock diets (Marston and DeRouchey, 2004). In 2020, each percent increase in the proportion of sprouted wheat resulted in a discount of $0.04 U.S. dollars/bu ($1.13/m^3) across all classes and subclasses of wheat (USDA, 2020).

In sprouted wheat, the activity of α-amylase increases which is responsible for starch breakdown (Benincasa et al., 2019). On average, α-amylase activity increases by 94% in sprouted wheat compared to non-sprouted wheat (Simsek et al., 2014). The starch in sprouted wheat is broken down into glucose which serves as the energy source for germination. As a result, sprouted wheat contains less total starch but more soluble starch than non-sprouted wheat. Świeca et al. (2017) observed that total starch content was reduced from 65.2% in wheat flour to 58.4% in sprouted wheat flour. However, the total proteins were not significantly different between sprouted and non-sprouted wheat flour. Ultimately, the effects on the nutrient composition are dependent on the proportion of sprouted vs non-sprouted kernels (Benincasa et al., 2019). Therefore, proximate and starch analysis should be performed to confirm nutrient profile for inclusion in swine diets.

Research evaluating sprouted wheat has focused on the percentage of sprouted to non-sprouted wheat and not the severity of sprouting. Murray and Huber (1968) observed weanling pigs fed increasing proportions of sprouted to non-sprouted wheat had similar ADG but poorer feed efficiency. The authors observed proportions of 20, 40, and 60% sprouted wheat resulted in 3.9, 6.8, and 7.8% poorer feed efficiency compared to pigs fed only non-sprouted wheat, respectively. In addition, Eide et al. (1979) observed pigs fed a proportion of 20% sprouted hard red spring wheat improved feed efficiency by 11% compared to a barley control diet with no effect on ADG. However, pigs fed a proportion of 40% sprouted hard red spring wheat had similar performance as the barley control diet. Therefore, the 20% sprouted hard red spring wheat diets still had greater energy density than the barley diet, while the 40% sprouted hard red spring diet was comparable to the barley diet. In addition, Eide et al. (1979) also observed pigs fed a proportion of 20 and 40% sprouted durum improved growth performance compared to the

barley control diet, with performance being most improved in the 20% sprouted durum diet. The 20% sprouted durum diet improved ADG and feed efficiency by 17 and 13%, respectively, whereas the 40% sprouted durum diet improved ADG and feed efficiency by 4 and 11%, respectively. Therefore, as the proportion sprouted to non-sprouted wheat increases, the energy value will decrease. However, sprouted wheat can still be fed effectively in swine diets if the energy density of the diet is considered. In addition, more severe sprouting will have a greater impact on energy and should be fed at lower proportions than moderately sprouted wheat.

Sprouted wheat can also have an impact on grinding characteristics. Particle size of sprouted wheat kernels will tend to be smaller than non-sprouted kernels. This is a result of more accumulation of fine particles and less coarse particles from grinding sprouted wheat (Fu et al., 2014; Dziki et al., 2015). Therefore, it may be beneficial to target a coarser particle size for sprouted wheat to reduce the number of fine particles. In addition, the energy requirements to obtain a specific particle size will be less when using sprouted wheat compared to non-sprouted wheat (Dziki and Lakowski, 2010). These modifications will result in more significant changes to the milling characteristics as severity of sprouting becomes greater.

If sprouted wheat is available and economically prices into a swine diet, it can be effectively utilized. Research suggests sprouted wheat contains lower concentrations of energy than non-sprouted wheat, therefore, depending on the proportion of sprouted to non-sprouted wheat, the energy value of wheat should be adjusted. The energy content of 10 to 40% sprouted wheat should be discounted by approximately 10%, whereas a proportion of sprouted wheat 40% or greater energy should be decreased by 15 to 20%. In addition, proximate analysis should be performed to account for any other nutrient changes from severe sprouting.

Low-test weight wheat

The test weight of wheat is a measure of grain density or the weight at a specific volume. Test weight in wheat is influenced by different classes/varieties, growing conditions, high moisture around harvest, insect damage, and sprout damage (Wang and Fu, 2020). Freezing when the wheat kernel is still developing is a major cause of low-test weight wheat (Jones et al., 2019). The test weight of wheat will affect its grade and ultimately its end use. Once test weight begins to fall below 60 lb/bu (772 kg/m^3), the U.S. grade will progressively worsen (USDA, 2014). Although, hard red spring and white club wheat (a subclass of soft white wheat) may naturally have lighter test weights than other classes and subclasses.

The nutritive value of low-test weight wheat will be dependent on the severity of test weight reduction. Low-test weight wheat is generally characterized at or below 51 lb/bu (656 kg/m^3; Jones et al., 2019). As wheat kernels develop, protein is deposited first followed by starch. Therefore, if development is interrupted, the result is incomplete starch deposition and increased protein to starch ratio (Lalman and Highfill, 2002). Therefore, low-test weight wheat will have decreased energy density. Protein may also need to be adjusted because of the change in the protein to starch ratio, but data is not available to confirm this hypothesis. It is estimated the feeding value of wheat is 95% to that of corn when wheat test weight is between 45 and 51 lb/bu (579 and 656 kg/m^3). Hines and Pollman (1982) observed that decreasing wheat test weight from 59 to 45 lb/bu (759 to 579 kg/m^3) resulted in no differences in ADG, but 6 and 8% poorer feed efficiency for 51 and 45 lb/bu (656 and 579 kg/m^3) wheat, respectively. Therefore, feeding value of wheat is likely dependent on the severity of test weight reduction.

Mycotoxin-contaminated wheat

Mycotoxins are naturally occurring toxins produced from certain molds and fungi found on a variety of different crops. In wheat, the most common mycotoxins are classified into 1 of 7 groups which include: Aflatoxins, A-Trichothecenes, B-Trichothecenes, Fumonisins (FUM), Zearalenone (ZEN), Ochratoxin A (OTA), and ergot alkaloids (DSM, 2022). Deoxynivalenol (DON) is a B-Trichothecene which is most common in wheat. A ten-year survey conducted by Gruber-Dorninger et al. (2019) reported DON was present in 65% of wheat samples with a median concentration of 0.37 mg/kg. The authors also reported 28% of wheat samples contained both DON and ZEN. In addition, ZEN, FUM, aflatoxin, and OTA were present in 33, 14, 10, and 9% of wheat samples, respectively. The response in pigs to feeding wheat and wheat co-products containing mycotoxins will depend on the type of mycotoxin, concentration, presence of multiple mycotoxins, and stage of pig production.

The U.S. Food and Drug Administration's (FDA; 2010) guidance level for maximum DON contamination in wheat and wheat co-products intended for swine feeding is 5 mg/kg and that this grain does not exceed 20% of the diet corresponding to 1 mg/kg of DON in the complete diet. A simple linear regression was developed from collected publications to determine the effects of increasing DON level of the diet from DON-contaminated wheat on pig performance. Only diets with DON contamination above 1 mg/kg were included for the regression analysis based on the guidance levels of the FDA. For nursery pigs, each 1 mg/kg increase in DON contaminated wheat-based diet resulted in an 11 and 6% decrease in ADG and ADFI, respectively. For finishing pigs, each 1 mg/kg increase in a DON contaminated wheat-based diet resulted in a 2.6 and 2.7% decrease in ADG and ADFI, respectively. Therefore, these data would indicate that finishing pigs can tolerate higher DON levels in wheat-based diets compared to

nursery pigs. However, the finishing pig studies had longer feeding durations of DON-contaminated wheat diets than nursery diets. Therefore, pigs may have been able to adjust to DON-contaminated wheat resulting in smaller changes in growth performance which has been observed in corn-based diets (Becker et al., 2022). It is unclear why pig performance is more adversely affected early in feeding DON-contaminated diets rather than later but may be associated with initial immune activation increasing their maintenance requirement.

Although less prevalent, other mycotoxins can infect wheat grains and influence pig performance. Zearalenone is of most concern for breeding swine because of substantial effects on reproduction (Etienne and Jemmali, 1982; Jiang et al., 2011). Alm et al. (2006) observed oocyte degradation and reduced meiotic competence from feeding 0.235 mg/kg ZEN contaminated wheat to 6-month-old gilts. Thus, feeding ZEN contaminated wheat should not be fed in diets for the breeding herd. High OTA levels can decrease pig performance and also accumulate in the tissues because of its long biological half-life (Aoudia et al., 2009). Aoudia et al. (2009) observed pigs fed 0.12 mg/kg OTA contaminated wheat-based diets led to reduced growth performance and increased concentrations of OTA in plasma, kidney, and liver up to 4 weeks after diets were fed. Recently, contamination of FUM in cereal grains has been increasing (Rao et al., 2020). Bracarense et al. (2012) observed wheat-based diets contaminated with a combination of FUM and DON negatively affected intestinal morphology and cytokine expression, but wheat diets contaminated with FUM alone provided similar results to feeding the uncontaminated wheat diet. Ergot alkaloids can also be prevalent in wheat during years of heavy rainfall and wet soil during the flowering stage (Krskaab and Crews, 2008). Wheat is the second most susceptible cereal grain to ergot contamination, only preceded by rye (Coufal-Majewski et al., 2016). Oresanya et al. (2003) observed that increasing ergot-contaminated wheat-based diets

up to 20.8 mg/kg linearly decreased BW by 24%, ADG by 37%, ADFI by 10%, and G:F by 29% in nursery pigs. Most of the previously mentioned mycotoxins have guidance levels set by either the FDA or European Commission (2006) that should be referenced when feeding mycotoxin contaminated wheat. However, aflatoxin is the only mycotoxin that has a mandatory action level set by the FDA. The FDA requires wheat or wheat co-products intended for swine diets to contain less than 20 µg/kg and it cannot be blended to decrease the concentration (FDA, 2000). Therefore, any wheat or wheat co-product that exceeds this action level must not be used or sold.

Wheat co-products are also a potential opportunity for mycotoxins to contaminate swine diets. The level of mycotoxin contamination within the complete diet will be dependent on the level of contamination of the wheat co-product. However, wheat co-products are typically fed at a lower inclusion than wheat grain and therefore will have less impact of pig production depending on the level of contamination. A survey conducted by Gott et al. (2019) revealed 91.8% of wheat middlings samples collected contained at least one mycotoxin with the most common being DON and ergot alkaloids. Among positive wheat middlings samples, the mean concentration of DON and ergot alkaloids was 1.9 mg/kg and 0.5 mg/kg, respectively. Therefore, mycotoxin contamination of wheat co-products should not be overlooked as a potential method to contaminate the complete diet.

Mycotoxin-contaminated wheat can lead to economic losses in pig production depending on the class of mycotoxins and their concentration. To prevent mycotoxin contamination after harvest, ensure proper storage of wheat to avoid moisture accumulation. Organic acids can be used to prevent mold growth. Propionic, acetic, and formic acid has been observed to have high inhibition against certain molds, whereas citric acid may help to a lesser degree (Hassan et al., 2012). If mycotoxins are found to be present in wheat, there are several methods to reduce its

harmful effects. Contaminated wheat grains can be cleaned to remove fines and damaged kernels that often contain higher mycotoxin concentrations. Optical NIRS grain sorters are an effective method to lower mycotoxin levels in contaminated wheat. Commercial mycotoxin mitigants can be used to lessen the negative effects on pig growth. Blend contaminated wheat with uncontaminated wheat at a ratio that will fall below the allowance levels established by the FDA or European Commission when allowed. Mycotoxin-contaminated wheat should only be used as a partial grain source in swine diets. The last precaution would be to avoid using mycotoxin contaminated wheat for nursery and breeding herd diets and fed only to finishing pigs.

Sprouting, low-test weight, and mycotoxin-contamination can be caused by many factors, with high-moisture field conditions most frequently associated. Therefore, wheat produced in high moisture environments is more likely to become off-quality. While sprouted and low-test weight wheat can still have a negative effect on performance, research suggests they can be fed effectively in swine diets as long as energy is adjusted accordingly depending on the severity of damage and proportion fed. High levels of mycotoxin contamination would likely lead to more severe reductions in performance of pigs compared to sprouted and low-test weight wheat. Therefore, mycotoxin contamination should be of most importance when evaluating off-quality wheat. Level and type of mycotoxin present in wheat will ultimately determine its application in swine diets.

Feeding Wheat Co-Products

Co-product production

During the milling process to produce wheat flour, the wheat kernel is separated to obtain the nutritional rich components. The wheat kernel is mainly comprised of bran (protective outer layer), endosperm (nutritional reserves for germination), and germ (reproductive component;

Campbell, 2007). The endosperm is rich in starch and protein that is extracted for flour milling. Once the endosperm is separated, the germ, bran, and some residual endosperm components remain, which make up the various wheat co-products (Figure 6). Wheat bran is defined as the coarse outer covering of the wheat kernel as separated from cleaned and scoured wheat in the usual process of commercial milling (AAFCO, 2023). Other wheat co-products such as wheat middlings (often referred to as wheat midds), wheat shorts, wheat millrun, and wheat red dog are produced when wheat bran is combined with wheat germ, wheat flour, and some offal from the "tail of the mill" (AAFCO, 2023). However, depending on the combination and concentrations of these fractions the co-product classification changes (Huang et al., 2012). The Association of American Feed Control Officials (2023) classifies each co-product based on crude fiber (CF) content in which wheat middlings, millrun, shorts and red dog must contain no more then 11, 9.5, 7 and 4% CF, respectively. The definition provided by AAFCO (2023) for wheat bran does not provide a CF classification as it does with the other co-products, but it can be assumed that CF should be greater than 11% CF since it only contains the outer covering of the wheat kernel and no germ or endosperm components.

Although each wheat co-product classification is outlined by AAFCO (2023), there are discrepancies of terminology depending on geographical location. Wheat millrun is commonly characterized in Canadian literature, but little is available outside of that region. High variability is often associated with wheat millrun contributing to its classification difficulty. Yarlagadda and Lee (2008) utilized the terms wheat millrun and wheat midds interchangeably when referring to leftover materials after flour extraction. Similarly, there appears to be some discrepancies between wheat middlings and bran between North America and Europe (Espinosa et al., 2024). The reclassification process of some sources of co-products was based AAFCO (2023)

definitions of wheat co-products and nutrient outliers to correct these discrepancies in the current review.

Other wheat co-products exist outside of the flour milling industry such as wheat co-products from biofuel production. A review conducted by Rosenfelder et al. (2012) discusses additional wheat co-products such as wheat dried distillers grains. However, the current review focusses on wheat flour milling co-products. Wheat flour co-products are commonly characterized in literature focused on swine, but organization and discussion of their nutritional differences and application in swine diets is warranted.

Nutrient concentrations

Wheat co-product classification is based on the amount of bran, germ, and residual endosperm fractions present (Sarfaraz et al., 2017). Transitioning from wheat bran to middlings to millrun to shorts to red dog is associated with less bran and more germ and flour fractions. Starch concentration increases from each co-product to the next which results in similar reductions in other nutrient values, particularly fiber (Table 9).

For direct comparisons, wheat middlings are slightly greater in CP, EE, Ca, P, and starch than wheat bran. Wheat bran mostly consists of the fibrous outer covering of the wheat kernel which explains its high fiber and low starch content. The concertation of starch in wheat bran from the current review is lower than the NRC (2012), which is likely due to recent advances in starch extraction during flour milling (Jaworski et al., 2016). In the literature, wheat middlings and millrun were very similar in their mean nutrient values. The similarities may be a result of terminology differences from geographical locations and the two co-products may be nutritionally equivalent. Wheat shorts had lower nutrient concentrations in almost all categories compared to wheat middlings and millrun, but higher in starch content. Similarly, wheat red dog

had lower nutrient concentrations in almost all categories compared to wheat shorts, but higher in starch content. To our knowledge, this is the first review to summarize wheat shorts and red dog nutrient values based on current literature. A review by Rosenfelder et al. (2013) only characterized wheat bran and middlings, and the NRC (2012) only reports one citation for wheat shorts.

For AA profile levels, wheat middlings, shorts and millrun generally had higher levels than wheat bran and red dog. This would be explained by those having higher CP percentages. Wheat middlings mean values were similar to observations for wheat millrun; however only one journal article was obtained with the AA profile of wheat millrun. The Lys concentration of the various co-products was 0.74, 0.70, 0.62, 0.59 and 0.45% for millrun, middlings, bran, shorts, and red dog, respectively. When compared to other literature, wheat middlings AA profile is similar to values reported by the NRC (2012).

Standardized ileal digestibility of crude protein and amino acids

To our knowledge there is no published literature characterizing the SID of CP and AA for wheat millrun. We speculate that SID of CP and AA in wheat millrun is similar to wheat middlings because of their similarities in nutrient and AA content. The SID of CP for wheat bran, middlings, shorts, and red dog is 67.5, 70.6, 85.9, and 88.1%, respectively (Table 10). The SID of Lys for wheat bran, middlings, shorts, and red dog is 69.3, 70.2, 87.9, and 83.1%, respectively. Ten wheat middlings samples from Casas and Stein (2017) were removed from mean SID of CP and AA summarization because the author's reported samples were heat damaged from processing. Lysine is particularly sensitive to heat damage because of its free amino group which easily reacts with a reducing sugar to form Maillard reaction products and becomes unavailable to the pigs (Almeida et al., 2013). Therefore, processing of wheat co-

products should be monitored to avoid heat damage and negatively influence the digestibility of Lys and other AA in the co-products.

There appears to be a clear divide between SID of CP and AA of wheat bran/middlings and wheat shorts/red dog. Wheat bran and middlings have lower SID percentages than wheat shorts and red dog. The total SID of all indispensable AA for wheat bran and middlings was 74.0 and 76.9%, respectively, whereas the total SID of all indispensable AA for wheat shorts and red dog was 90.2 and 89.4%, respectively. Therefore, including wheat shorts or red dog will result in better digestible CP and AA in swine diets than wheat bran or middlings.

Wheat middlings had greater SID values of CP and all indispensable AA compared to wheat bran. When comparing wheat shorts and red dog, SID of CP was greater for wheat red dog than shorts. Wheat red dog was also greater in SID of Arg, Ile, Leu, Met, and Trp while wheat shorts was greater in SID of His, Lys, Phe, Thr, and Val. However, the greatest difference between the co-products for SID of all indispensable AA was only 4.8% units observed for Lys. There was very little information from publications collected on the SID of dispensable AA for wheat shorts and red dog.

While wheat co-products are lower in SID of CP and AA compared to wheat grain because of higher fiber, they still have a higher concentration of total CP and AA. This allows the opportunity to add more feed grade Lys and other AA because CP needed for non-essential AA production is met utilizing wheat co-products. Overall, wheat co-products may have lower SID of CP and AA compared to the wheat grain they originated from, but they are still a low-cost ingredient with relatively high CP and AA.

Phosphorus

Wheat co-products are excellent sources of P for swine diets. Wheat bran, middlings, and millrun P level were 0.99, 1.05, and 0.97%, respectively. Wheat bran, middlings, and millrun are at least 0.37% higher in P compared to other common co-products such as bakery meal, corn dried distillers grains, oat groats, and soybean hulls (NRC, 2012). Wheat is already a high P cereal grain, but when flour is extracted from wheat during the milling process P becomes more concentrated in the co-products resulting in increased P. The P concentration of wheat bran and middlings from the current review are similar to values reported by NRC (2012). The highest concentrations of intrinsic phytase are typically found in the bran of the wheat kernel (Cornell, 2012; Katamadze et al., 2023). Therefore, wheat bran and other co-products with higher proportions of bran will have higher intrinsic phytase activity. Feeding wheat bran, middlings, or millrun in swine diets will result in less needed supplemental inorganic P such as monocalcium or dicalcium phosphate and can reduce diet cost. Wheat shorts and red dog contain 0.40 and 0.30% P, respectively, which is similar to conventional wheat.

Energy

The energy concentrations of wheat co-products are dependent on the level of starch and overall fiber (Huang et al., 2014; Lyu et al., 2023). Across all co-products, wheat bran had the lowest mean DE and ME values of 2,796 and 2,636 kcal/kg DM, respectively (Table 11). Wheat red dog was highest among co-products with average DE and ME of 3,665 and 3,473 kcal/kg DM, respectively. There was only a single publication from Woyengo and Ziljstra (2021) that reported DE and ME of wheat millrun. Therefore, no mean energy values were developed for wheat millrun, but we speculate energy values would be similar to wheat middlings because of

similarities in nutrient profile. On average, mean ME across all co-products was determined to be 95% of mean DE in the current review.

When comparing mean energy values of wheat bran to middlings from publications collected, the mean difference in DE was 186 kcal/kg, whereas the difference in ME was 236 kcal/kg. This is likely due to the higher CP, starch, and fat associated with wheat middlings in combination with lower fiber content. In contrast, the difference in DE was 362 kcal/kg when comparing wheat middlings and shorts mean energy values, whereas the difference in ME was 280 kcal/kg. This again could be a result of nutrient differences between the two co-products, but also wheat shorts may have a slower passage rate from greater soluble fiber allowing for more nutrient absorption and less excretion in the feces than middlings (Wenk, 2001). When comparing wheat shorts and red dog, the difference between mean DE and ME for both was 321 kcal/kg.

In addition, reported DE, EE, starch, CP, and ADF from each publication were utilized to calculate NE of each co-product (Eq. 1-8; NRC, 2012). Higher DE, EE, and starch within a wheat co-product sample would correlate to greater NE values, whereas greater CP and ADF would correlate to lower NE values. Calculated NE of wheat co-product samples followed a similar pattern as mean DE and ME with bran being lowest and red dog being highest. On average, mean calculated NE across all co-products was 74% of ME. Average GE was similar across wheat bran, middlings, shorts, and red dog with an average value of 4,451 kcal/kg DM and a range of 208 kcal/kg.

Co-product influence on growth performance

The optimal wheat co-product level in a swine diet is a balance between diet cost, ingredient consistency, and growth performance. Co-products are often variable in nutrient

concentration that may lead to variation in pig performance. In addition, high inclusion of fibrous wheat co-products may require feeder adjustments because of potentially feed flowability issues. To have increased confidence in the co-product being evaluated or used, regular chemical analysis moisture, CP, fiber (CF, ADF, and NDF) is recommended to confirm the nutrient profile. Starch analysis may also be warranted particularly for wheat shorts and red dog because of their higher concentrations compared to the other wheat co-products. As previously described, the levels of fiber, protein, starch and other nutrients will impact how the co-product will optimize in inclusion rate and stage of production in a swine diet.

The majority of literature that focuses on pig performance from feeding wheat co-products consists of wheat bran and wheat middlings. Most wheat bran research has been conducted in nursery pigs to improve gastrointestinal health based on its fiber content. Wheat middlings research has been conducted in both nursery pigs and finishing pigs. Its application in finishing diets has gained attention because of it being a more economical source of nutrients compared to the higher cost of grain and intact protein ingredients commonly used in swine diets. Wheat millrun has literature in both nursery and finishing pigs, however it is less prevalent than wheat bran and middlings. There is little research available on wheat shorts and published studies that are available were conducted prior to 1982. To our knowledge, there are no published studies evaluating the effects of wheat red dog on pig performance which warrants future attention.

Nursery pigs

When nursery diets are formulated to equal energy levels, research indicates diets containing wheat bran can improve feed efficiency without dietary antibiotic use. Gasa et al. (2010), Molist et al. (2011), and Zhao et al. (2018) observed nursery pigs fed wheat bran

improved feed efficiency by 8.8, 8.1, and 6.1%, respectively compared to a control diet without wheat bran. In addition, Gasa et al. (2010) also observed an increase in ADG from pigs fed wheat bran compared to a control diet. Gasa et al. (2010) and Molist et al. (2011) fed wheat bran at 4% of the diet, whereas Zhao et al. (2018) fed 5% wheat bran. Improvements in feed efficiency may be a result of improved gastrointestinal health of young pigs which is discussed later in the review. However, across all three studies, chemical analysis showed wheat bran diets were greater in GE than control diets that likely contributed to the feed efficiency response. Batson et al. (2021) and Laskoski et al. (2021) observed growth performance was comparable between pigs fed a control diet and a diet with 4% wheat bran in non-isocaloric diets. Therefore, research has shown wheat bran can be included at 4 to 5% of the diet without negatively affecting nursery pig performance whether diets are isocaloric or not.

De Jong et al. (2014) evaluated the effects of added wheat middlings on nursery pig performance. In these studies, all diets were not formulated to be isocaloric. In summary of the four experiments, increasing added wheat middlings decreased growth performance with 5, 10, 15, and 20% wheat middlings decreasing ADG by 0.6, 1.7, 2.1, and 5.0%, respectively, and ADFI by 1.4, 1.7, 2.6, and 1.5%, respectively. Feed efficiency was relatively unaffected by increasing added wheat middlings until 20% inclusion which resulted in a 4.6% reduction in G:F. Shaw et al. (2002) also fed 10% wheat middlings to nursery pigs and observed similar reductions in growth performance as De Jong et al. (2014). However, Shaw et al. (2002) diets were formulated to be isocaloric unlike De Jong et al. (2014).

Garcia et al. (2015) conducted a study with increasing wheat millrun in addition to increasing levels of canola oil to balance dietary energy. The authors reported increasing wheat millrun from 0 to 20% of the diet led to a linear improvement in feed efficiency. However,

chemical analysis revealed energy density of the diet also increased, thus indicating millrun, canola oil or both were undervalued in energy at formulation. However, if wheat millrun is added to a nursery diet without balancing energy it will likely lead to a similar response to wheat middlings because of their nutrient similarities. To our knowledge, there are no studies evaluating nursery pig performance feeding wheat shorts or red dog. However, these co-products are higher in energy and may be able to be incorporated with similar or less impact on performance.

Finishing pigs

Nursery pig's appetite limits their ability to consume enough feed to meet their growth potential. However, finishing pig's feed intake exceeds their ability to meet their growth potential. Therefore, finishing pig growth performance will likely be more severely affected with increasing levels of wheat co-products than in nursery pigs because of the reduction in energy density of the diet. Du et al. (2023) fed increasing wheat bran (0, 7, 14, and 21%) to finishing pigs and observed significant reductions in ADG and ADFI. All three levels of wheat bran inclusion led to similar reductions in ADG and ADFI by approximately 20 and 15%, respectively. Anguita et al. (2007) also observed reductions in ADFI from feeding 10% wheat bran, but they also observed an improvement in feed efficiency. However, the authors added soy oil to balance energy which resulted in the wheat bran diet having 95 kcal/kg greater GE based on chemical analysis.

Similar to nursery pigs, as inclusion rate of wheat middlings increase, performance decreases because of reductions in energy density of the diet. Salyer et al. (2012) observed 10 and 20% added wheat middlings decreased ADG by 3.2 and 4.5%, respectively, and G:F by 2.7 and 4.2%, respectively, although a constant SID Lys:ME/Mcal ratio was maintained. In contrast,

Shaw et al. (2002) and Wu et al. (2016) did not observe any growth differences from feeding wheat middlings at 30 and 15% of the diet, respectively when energy was balanced. In non-isocaloric diets, Stewart et al. (2013) observed 30% added wheat middlings lead to 16.0 and 11.2% poorer ADG and feed efficiency, respectively compared to a control diet. Salyer et al. (2012), Stewart et al. (2013), and Wu et al. (2016) also observed decreased carcass weight and yield from feeding diets containing wheat middlings, however Shaw et al. (2002) did not observe any differences in carcass characteristics.

Wheat millrun appears to lead to similar reductions in performance as wheat middlings. Nortey et al. (2007) observed pigs fed 20 and 40% wheat millrun decreased ADG by 2.9 and 6.9%, respectively, and G:F by 4.9 and 7.3%, respectively in energy balanced diets. In non-isocaloric diets, Kpogo et al. (2021) observed 30% added wheat millrun reduced ADG and feed efficiency by 2.7 and 5.1%, respectively. The authors also reported decreased back fat depth from feeding 30% wheat millrun. However, unlike wheat middlings, the authors observed an increase in carcass yield with feeding wheat millrun.

Available research suggests wheat shorts can be fed at a higher inclusion compared to previously mentioned wheat co-products due to their higher energy. Young (1980) observed pigs fed 32.2% wheat shorts had comparable performance to pigs fed a control diet with no added wheat shorts in non-isocaloric diets. However, when wheat shorts were fed at 64.4 and 96.6% of the diet performance decreased. To our knowledge, there are no published growth performance studies on wheat red dog in finishing pigs.

Sows

In sow diets, wheat co-product application may be most beneficial during gestation. Limit feeding during gestation can cause aggressive behavior because sows may be unfulfilled from

the amount of feed they receive (Meunier-Salaün et al., 2001). Incorporating wheat co-products during gestation may help to increase satiety in sows to reduce aggressive behavior (Guillemet et al., 2007). In addition, wheat co-products can be fed in diets for replacement gilts to limit ADG and reduce feed cost until breeding ultimately lowering the cost of non-productive days. Wheat co-product inclusion may be more challenging in lactating sow diets because of their lower energy content, but if fed at lower levels they may be able to be used without diminishing feed intake. Shang et al. (2019) observed sows fed wheat bran in late gestation (30% of the diet) and lactation (15% of the diet) led to a numeric increase in sow feed intake. In this study, the gestation diet containing wheat bran was lower in energy but fed to achieve equal DE intake across all treatments, whereas the lactation diets were balanced for energy and fed *ad libitum*. The authors also observed piglets from the sows fed wheat bran had numerically greater weaning weight and ADG. Renaudeau et al. (2003) observed sows fed lactation diets containing a combination of wheat bran and wheat middlings at 47.9% of the diet resulted in greater body weight and backfat loss, but piglets had increased ADG and weight at weaning even though diets were formulated to be 263 kcal/kg less than the control diet. The authors speculated the improved litter growth was because of increased milk production, but mechanisms behind increased milk production were unclear. Young and King (1981) observed sows fed diets completely comprised of wheat shorts during gestation had 1.2 more pigs weaned, but 0.08 kg lighter birth weight than sows fed a corn-soybean meal gestation diet. To our knowledge there is no research evaluating wheat millrun or red dog in sow diets and the effect it has on sow and litter performance.

High fiber wheat co-products such as wheat bran, middlings, and millrun can be fed in all stages of production, but performance may be decreased if not adjusted for energy. Wheat fiber

reduces overall organic matter digestibility which contributes to reduced performance (Son et al., 2023). Also, their higher CP can replace intact protein sources and increased P concentration can lower the amount of added inorganic P to the diet. Wheat shorts and red dog may be included at higher levels without as much impact on performance as other wheat co-products. Overall, wheat co-products can be fed effectively in all stages of production.

Co-product fiber and gastrointestinal health

The studies discussed in the current review focus on wheat co-product's influence on gastrointestinal health and do not include diets with antibiotics. It is important to note that dietary antibiotics use will affect the response in pigs when used in combination or comparing against wheat co-products. Antibiotic use is swine diets is becoming less frequent because of the occurrence antibiotic resistance. This section provides an insight into how wheat co-products can influence gastrointestinal health in diets without antibiotics.

Historically, high fiber ingredients were considered to have a lower nutritional value because of their lower energy or amino acid concentrations (Li et al., 2021). More recently, high fiber ingredients are gaining attention because of their ability to lower diet cost and provide gastrointestinal benefits. Based on summarized mean nutrient values from the current review, wheat bran, middlings, and millrun are good sources of insoluble fiber containing 38.9, 33.7, and 35.5%, respectively. Although not as high as the other wheat co-products, wheat shorts and red dog still contain 15.2 and 12.8% insoluble fiber, respectively. In addition, wheat shorts also contain 4.1% soluble fiber.

Of all wheat co-products, wheat bran has the highest NDF, TDF, and insoluble fiber. Wheat bran has gained attention because of its potential benefits on gut health in weaned pigs (Koo et al., 2017). Wheat bran's high insoluble fiber can act to inhibit pathogen adhesion by

providing an alternative adhesion site (Molist et al., 2014). Molist et al. (2010) observed 4% wheat bran significantly reduced ileal *E. coli* concentration regardless of wheat bran particle size in nursery pigs challenged with *E. coli* $K88^{+}$. The authors also observed pigs fed coarse wheat bran improved fecal consistency. In addition to wheat bran, wheat middlings are also a good source of insoluble fiber. Although not significant, Berrocoso et al. (2015) observed numeric benefits to feeding 2.5 and 5% wheat middlings in nursery pig diets compared to other fiber sources at the same inclusion rate under poor hygienic conditions. The authors observed wheat middlings had numerically improved villus height:crypt depth ratio and post-weaning diarrhea presence compared to straw, oat hulls, and sugar beet pulp. Therefore, high insoluble fiber wheat co-products at 4 to 5% of the diet may be fed in nursery pig diets to improve gastrointestinal health parameters especially during enteric challenges.

High insoluble fiber in wheat co-products can also act to enhance proliferation of beneficial bacteria in the gastrointestinal tract of pigs (Heo et al., 2013). Wang et al. (2023) observed non-pregnant sows fed 20% finely ground wheat bran was able to increase the abundance of Lactobacillaceae and concentrations of their metabolites such as short chain fatty acids in the ileum. In addition, Leblois et al. (2017) observed 7% wheat bran fed to sows during gestation and lactation was able to modulate the piglet's microbiota. Similar findings were reported by Li et al. (2023) where growing pigs fed 16% wheat bran had increased abundance of Streptococcus and Prevotellaceae bacteria which resulted in increased total antioxidant capacity of blood serum. Streptococcus bacteria contribute to short chain fatty acid production, while Prevotellaceae maintain intestinal health and enhance immune and antioxidant function (Downes et al., 2013; Zhang et al., 2021). Liu et al. (2023) also observed improved serum antioxidant capacity and cecal short chain fatty acid concentration in addition to improved gut morphology

from pigs fed 17% wheat bran fiber. Little data is available on the effect other wheat co-products have on the microbiome. However, wheat middlings and millrun may lead to similar responses as wheat bran because of the high insoluble to soluble fiber ratio characteristics.

Conclusion

Wheat and wheat co-products can offer certain benefits in swine diets such as high CP, AA, and P. In addition, wheat and wheat co-products are particularly appealing for pelleted diets because of improvements in pellet quality. When formulating with wheat and wheat co-products, it is important to know the class of wheat or the wheat co-product classification for accurate nutrient formulation. The current review provides a summarization of nutritional aspects associated with wheat and wheat co-products, where variation may occur, and their implementation into swine diets.

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Table 5.1 United States wheat classification[1]

Class:	Hard red winter	Hard red spring	Soft red winter	Hard white	Soft white	Durum
Protein range (%) 12% moisture basis	10.0 – 13.0	12.0 – 15.0	8.5 – 10.5	10.0 – 14.0	8.5 – 10.5	12.0 – 15.0
Growing region (top 5 producing)	Kansas Oklahoma Montana South Dakota Texas	North Dakota Minnesota Montana Idaho South Dakota	Illinois Ohio Kentucky Tennessee Missouri	Kansas Nebraska Colorado Utah California	Washington Idaho Oregon Michigan Utah	North Dakota Montana Arizona California Idaho
Planting season	Fall	Spring	Fall	Fall or spring	Fall or spring	Spring
Harvest season	Summer	Fall	Summer	Summer or fall	Summer or fall	Fall
Application	Asian noodles Hard rolls Flat breads Flour Cereal	Hearth breads Rolls Croissants Bagels Pizza crust	Cookies Crackers Pretzels Pastries Flat bread	Asian noodles Pan breads Flat breads Whole wheat	Cakes Patries Asian noodles Flat breads	Pasta Breads
Production (million metric tons)	15.93	11.23	11.98	0.51	5.98	1.56
Production (% of all wheat)	33.8	23.8	25.4	1.1	12.7	3.3
Sub-classes	None	Dark northern spring Northern spring Red spring	None	None	Soft white White club Western white	Hard amber durum Amber durum Durum

[1] USDA, 2023 and U.S. Wheat Associates, 2023.

Table 5.2 USDA grades and grade requirements for all classes and subclasses of wheat[1]

	Grade U.S. Nos.				
Grading factors	1	2	3	4	5
Minimum limits (lb/bu)					
Test weight					
Hard red spring wheat or white club wheat	58.0	57.0	55.0	53.0	50.0
All other classes and subclasses	60.0	58.0	56.0	54.0	51.0
Maximum limits (%)					
Damaged kernels					
Heat (part of total)	0.2	0.2	0.5	1.0	3.0
Total	2.0	4.0	7.0	10.1	15.0
Foreign material	0.4	0.7	1.3	3.0	5.0
Shrunken and broken kernels	3.0	5.0	8.0	12.0	20.0
Total[2]	3.0	5.0	8.0	12.0	20.0
Wheat of other classes	10.0	10.0	10.0	10.0	10.0
Contrasting classes	1.0	2.0	3.0	10.0	10.0
Total[3]	3.0	5.0	10.0	10.0	10.0
Stones	0.1	0.1	0.1	0.1	0.1
Maximum count limits[4]					
Animal filth	1	1	1	1	1
Castor beans	1	1	1	1	1
Crotalaria seeds	2	2	2	2	2
Glass	0	0	0	0	0
Stones	3	3	3	3	3
Unknown foreign substances	3	3	3	3	3
Total[5]	4	4	4	4	4
Insect-damaged kernels in 100 g	31	31	31	31	31

[1] USDA, 2014. Does not include mixed wheat

[2] Includes damaged kernels, foreign material, shrunken and broken kernels

[3] Includes contrasting classes

[4] Any wheat purchase that exceeds the maximum count limit is not eligible for a U.S. grade.

[5] Includes any combination of animal filth, castor beans, crotalaria seeds, glass, stones, or unknown foreign substance.

Table 5.3 Chemical composition and standardized ileal digestibility (SID) of nutrients, amino acids, and energy content in wheat (as-fed basis)[1]

		Chemical composition (%)				SID (%)		
Item	N[2]	Mean ± SD	Min	Max	N[2]	Mean ± SD	Min	Max
Nutrient composition, %								
DM	36	88.9 ± 1.62	86.5	92.9				
CP	55	12.6 ± 1.75	8.5	17.6	28	87.2 ± 4.75	75.0	90.4
EE	52	1.7 ± 0.55	0.7	3.8				
Ash	49	1.6 ± 0.24	0.7	2.1				
ADF	49	2.8 ± 0.77	0.8	5.8				
NDF	49	11.7 ± 2.79	8.1	23.1				
TDF	16	10.9 ± 1.68	9.2	15.2				
Ca	30	0.07 ± 0.046	0.02	0.19				
P	30	0.27 ± 0.104	0.07	0.45				
Starch	39	57.7 ± 5.99	30.3	64.4				
Indispensable AA, %								
Arg	43	0.56 ± 0.055	0.47	0.71	30	88.4 ± 4.51	80.6	99.8
His	43	0.34 ± 0.082	0.22	0.53	30	88.2 ± 4.51	80.9	94.1
Ile	45	0.43 ± 0.066	0.30	0.57	30	87.1 ± 4.25	78.4	94.0
Leu	43	0.84 ± 0.141	0.53	1.18	29	88.1 ± 3.91	80.5	94.8
Lys	45	0.38 ± 0.059	0.30	0.53	30	76.8 ± 7.95	63.7	89.9
Met	45	0.19 ± 0.028	0.14	0.26	30	88.3 ± 3.39	82.9	94.5
Phe	43	0.58 ± 0.108	0.42	0.84	30	89.2 ± 4.20	82.3	96.0
Thr	45	0.35 ± 0.041	0.27	0.44	30	80.9 ± 5.80	65.4	90.8
Trp	43	0.15 ± 0.028	0.08	0.21	28	86.5 ± 4.92	76.8	94.6
Val	45	0.56 ± 0.113	0.39	0.83	30	85.0 ± 4.41	75.1	92.9
Dispensable AA, %								
Ala	39	0.47 ± 0.079	0.37	0.64	29	80.0 ± 7.04	61.7	90.8
Asp	39	0.63 ± 0.071	0.52	0.79	29	80.7 ± 6.65	66.8	91.5
Cys	39	0.31 ± 0.065	0.20	0.46	29	89.2 ± 3.85	78.3	95.1
Glu	39	3.45 ± 0.582	2.54	5.16	29	94.8 ± 2.15	88.9	97.5
Gly	38	0.50 ± 0.050	0.41	0.64	28	85.3 ± 8.08	63.8	96.4
Pro	38	1.28 ± 0.254	0.84	1.93	27	109.9 ± 45.47	71.7	328.0
Ser	39	0.53 ± 0.083	0.25	0.71	29	89.3 ± 3.59	80.3	94.7
Tyr	28	0.16 ± 0.125	0.02	0.41	17	90.9 ± 6.51	77.1	99.5
Energy Content, kcal/kg DM								
GE	45	4,401 ± 100.2	3,954	4,604				
DE	27	3,900 ± 144.5	3,456	4,126				
ME	28	3,785 ± 132.8	3,353	3,975				

Calculated NE[3]	15	2,786 ± 60.0	2,672	2,860

[1] Literature Cited: Abelilla and Stein, 2019; Bloxham et al., 2018; Bolarinwa and Adeola, 2016; Cervantes-Pahm et al., 2014; De Jong et al., 2016; Eklund et al., 2014; Fan et al., 2017; Heyer et al., 2022; Jaworski et al., 2015; Jeon et al., 2019; Koo et al., 2018; Lee et al., 2020; Le et al., 2016; McGhee and Stein, 2018; McGhee and Stein, 2020; Oliveria et al., 2020; Pan and An, 2020; Park et al., 2016; Rodriguez et al., 2020; Rosenfelder et al., 2015; Schemmer et al., 2020; She et al., 2015; Tan et al., 2021; Wang et al., 2018; Xie et al., 2017; Zhang et al., 2020; Zhao et al., 2019; Zhou et al., 2016.

[2] Number of samples of wheat from collected publications utilized for mean values.

[3] Calculated using Eq. 1-8 from NRC (2012). Net energy (kcal/kg DM) = (0.700 × DE) + (1.61 × EE) + (0.48 × Starch) – (0.91 × CP) – (0.87 × ADF).

Table 5.4 Simple linear regression to predict amino acids concentration using the crude protein content in wheat[1]

Item	N[2]	Intercept	Slope (CP)	R-Square	Standard Error	*P* =
Indispensable AA						
Arg	43	0.273	0.023	0.598	0.036	< 0.001
His	43	-0.059	0.032	0.506	0.058	< 0.001
Ile	45	0.042	0.031	0.724	0.035	< 0.001
Leu	43	0.207	0.051	0.439	0.107	< 0.001
Lys	45	0.145	0.019	0.347	0.048	< 0.001
Met	45	0.031	0.013	0.700	0.016	< 0.001
Phe	43	-0.037	0.049	0.697	0.060	< 0.001
Thr	45	0.112	0.019	0.711	0.022	< 0.001
Trp	43	0.027	0.010	0.435	0.021	< 0.001
Val	45	0.044	0.041	0.438	0.085	< 0.001
Dispensable AA						
Ala	39	0.048	0.034	0.522	0.055	< 0.001
Asp	39	0.245	0.031	0.542	0.049	< 0.001
Cys	39	-0.009	0.026	0.432	0.049	< 0.001
Glu	39	-0.308	0.306	0.759	0.289	< 0.001
Gly	38	0.173	0.027	0.644	0.030	< 0.001
Pro	38	-0.215	0.122	0.647	0.153	< 0.001
Ser	39	0.105	0.035	0.471	0.061	< 0.001
Tyr	28	0.412	-0.020	0.074	0.122	0.161

[1] Literature Cited: Abelilla and Stein, 2019; Bloxham et al., 2018; Bolarinwa and Adeola, 2016; Cervantes-Pahm et al., 2014; De Jong et al., 2016; Eklund et al., 2014; Fan et al., 2017; Heyer et al., 2022; Jaworski et al., 2015; Jeon et al., 2019; Koo et al., 2018; Lee et al., 2020; Le et al., 2016; McGhee and Stein, 2018; McGhee and Stein, 2020; Oliveria et al., 2020; Pan and An, 2020; Park et al., 2016; Rodriguez et al., 2020; Rosenfelder et al., 2015; Schemmer et al., 2020; She et al., 2015; Tan et al., 2021; Wang et al., 2018; Xie et al., 2017; Zhang et al., 2020; Zhao et al., 2019; Zhou et al., 2016.

[2] Number of samples of wheat from collected publications utilized for mean values.

Table 5.5 Simple linear regression to predict content of standardized ileal digestible (SID) amino acids in relation to crude protein of wheat[1]

Item	N[2]	Intercept	Slope (CP)	R-Square	Standard Error	*P* =
Indispensable AA						
Arg	30	0.182	0.026	0.689	0.032	< 0.001
His	30	-0.165	0.038	0.588	0.058	< 0.001
Ile	30	-0.028	0.032	0.707	0.038	< 0.001
Leu	29	-0.165	0.074	0.758	0.069	< 0.001
Lys	30	-0.024	0.026	0.362	0.063	< 0.001
Met	30	0.012	0.013	0.735	0.014	< 0.001
Phe	30	-0.094	0.049	0.682	0.061	< 0.001
Thr	30	0.042	0.019	0.701	0.023	< 0.001
Trp	28	-0.017	0.012	0.581	0.018	< 0.001
Val	30	-0.113	0.047	0.611	0.068	< 0.001
Dispensable AA						
Ala	29	-0.077	0.037	0.505	0.066	< 0.001
Asp	29	0.043	0.038	0.513	0.068	< 0.001
Cys	29	-0.033	0.025	0.476	0.048	< 0.001
Glu	29	-0.396	0.299	0.795	0.276	< 0.001
Gly	28	0.038	0.032	0.534	0.049	< 0.001
Pro	27	0.395	0.080	0.126	0.394	0.069
Ser	29	0.116	0.029	0.480	0.055	< 0.001
Tyr	17	0.160	-0.002	0.001	0.107	0.926

[1] Literature Cited: Cervantes-Pahm et al., 2014; Lee et al., 2020; McGhee and Stein, 2018; Oliveria et al., 2020; Rodriguez et al., 2020; Rosenfelder et al., 2015; Tan et al., 2021; Wang et al., 2018; Zhao et al., 2019.

[2] Number of samples of wheat from collected publications utilized for mean values.

Table 5.6 Nutrient and amino acid composition and standardized ileal digestibility (SID) of wheat compared to corn (as-fed basis)[1]

	Chemical composition (%, Mean ± SD)				SID (%, Mean ± SD)			
Item	N[2]	Wheat	Corn	Ratio	N[2]	Wheat	Corn	Ratio
Nutrient composition, %								
DM	16	89.1 ± 1.62	88.0 ± 1.22	1.01				
CP	16	12.3 ± 1.85	7.9 ± 1.36	1.56	7	84.6 ± 6.92	81.8 ± 10.13	1.03
EE	15	2.1 ± 0.66	3.2 ± 0.57	0.66				
Ash	15	1.7 ± 0.22	1.3 ± 0.25	1.31				
ADF	11	3.0 ± 0.50	2.5 ± 0.73	1.20				
NDF	11	12.8 ± 3.10	10.3 ± 3.22	1.24				
Ca	6	0.11 ± 0.063	0.06 ± 0.060	1.83				
P	6	0.38 ± 0.055	0.24 ± 0.028	1.58				
Starch	9	56.7 ± 5.09	60.0 ± 4.87	0.95				
Indispensable AA, %								
Arg	10	0.56 ± 0.063	0.33 ± 0.019	1.70	8	86.1 ± 4.37	86.9 ± 9.35	0.99
His	10	0.27 ± 0.052	0.21 ± 0.020	1.29	8	85.4 ± 3.20	84.8 ± 4.66	1.01
Ile	10	0.41 ± 0.059	0.26 ± 0.031	1.58	8	82.7 ± 3.84	82.4 ± 7.12	1.00
Leu	10	0.72 ± 0.089	0.81 ± 0.108	0.89	7	84.6 ± 3.25	88.1 ± 4.57	0.96
Lys	10	0.37 ± 0.044	0.25 ± 0.029	1.48	8	72.5 ± 5.66	74.7 ± 10.59	0.97
Met	10	0.19 ± 0.027	0.15 ± 0.012	1.27	8	85.8 ± 2.30	88.4 ± 5.14	0.97
Phe	10	0.53 ± 0.096	0.39 ± 0.131	1.36	8	85.9 ± 2.98	85.9 ± 4.70	1.00
Thr	10	0.34 ± 0.041	0.26 ± 0.026	1.31	8	75.8 ± 6.21	75.8 ± 9.42	1.00
Trp	9	0.14 ± 0.024	0.06 ± 0.004	2.33	7	83.8 ± 4.52	74.3 ± 9.78	1.13
Val	10	0.51 ± 0.061	0.35 ± 0.037	1.46	8	79.9 ± 3.67	80.5 ± 6.71	0.99
Dispensable AA, %								
Ala	10	0.42 ± 0.039	0.52 ± 0.050	0.81	8	74.2 ± 6.71	84.4 ± 6.83	0.88
Asp	10	0.61 ± 0.067	0.50 ± 0.066	1.22	8	75.4 ± 5.70	80.9 ± 6.86	0.93
Cys	10	0.26 ± 0.020	0.17 ± 0.032	1.53	8	85.1 ± 3.56	80.2 ± 6.59	1.06
Glu	10	3.19 ± 0.668	1.28 ± 0.130	2.49	8	92.8 ± 1.66	87.2 ± 4.55	1.06
Gly	9	0.46 ± 0.018	0.29 ± 0.016	1.59	7	82.2 ± 12.08	80.2 ± 17.95	1.02
Pro	9	1.10 ± 0.231	0.62 ± 0.061	1.77	6	114.5 ± 22.77	99.8 ± 53.20	1.15
Ser	10	0.49 ± 0.077	0.33 ± 0.022	1.48	8	85.7 ± 2.71	83.5 ± 6.98	1.03
Tyr	7	0.29 ± 0.058	0.21 ± 0.020	1.38	4	84.6 ± 5.08	80.4 ± 4.31	1.05
Energy content (kcal/kg DM)								
GE	6	4,404 ± 174.9	4,418 ± 181.5	1.00				
DE	6	3,824 ± 218.1	3,882 ± 212.8	0.99				
ME	6	3,690 ± 206.7	3,780 ± 199.8	0.98				

[1] Mean values for each comparison are sourced from articles that have direct comparisons of wheat and corn. Literature cited: Abelilla and Stein, 2019; Cervantes-Pahm et al., 2014; Fan et al., 2017; Jaworski et al., 2015; Jeon et al., 2019; McGhee and Stein, 2018; McGhee and Stein 2020; Oliveria et al., 2020; Pan and An, 2020; Park et al., 2016; Rodriguez et al., 2020; She et al., 2015; Tan et al., 2021; Xie et al., 2017; Zhang et al., 2020.

[2] Number of wheat/corn comparisons from collected publications.

Table 5.7 Nutrient and amino acid composition and standardized ileal digestibility (SID) of wheat compared to barley (as-fed basis)[1]

	Chemical composition (%, Mean ± SD)				SID (%, Mean ± SD)			
Item	N[2]	Wheat	Barley	Ratio	N[2]	Wheat	Barley	Ratio
Nutrient composition, %								
DM	12	88.8 ± 1.60	88.9 ± 1.68	1.00				
CP	12	12.5 ± 2.34	11.2 ± 1.56	1.12	4	86.4 ± 7.59	80.7 ± 6.16	1.07
EE	11	1.9 ± 0.86	1.9 ± 0.56	1.00				
Ash	11	1.5 ± 0.34	1.9 ± 0.58	0.79				
ADF	11	3.1 ± 0.96	5.7 ± 2.07	0.54				
NDF	11	14.1 ± 4.06	19.0 ± 5.08	0.74				
Ca	7	0.05 ± 0.039	0.07 ± 0.033	0.71				
P	7	0.34 ± 0.038	0.33 ± 0.027	1.03				
Starch	7	58.0 ± 3.82	55.9 ± 6.34	1.04				
Indispensable AA, %								
Arg	8	0.58 ± 0.078	0.52 ± 0.067	1.12	4	92.3 ± 5.69	85.1 ± 4.40	1.08
His	8	0.29 ± 0.064	0.25 ± 0.037	1.16	4	86.5 ± 3.11	82.8 ± 2.49	1.04
Ile	8	0.44 ± 0.085	0.40 ± 0.075	1.10	4	84.3 ± 3.77	78.1 ± 4.14	1.08
Leu	8	0.77 ± 0.173	0.74 ± 0.171	1.04	3	84.8 ± 3.53	81.7 ± 3.59	1.04
Lys	8	0.39 ± 0.052	0.43 ± 0.058	0.91	4	77.6 ± 4.97	72.6 ± 6.08	1.07
Met	8	0.20 ± 0.041	0.18 ± 0.028	1.11	4	87.2 ± 2.54	80.7 ± 4.66	1.08
Phe	8	0.58 ± 0.139	0.58 ± 0.120	1.00	4	86.3 ± 2.59	82.9 ± 3.28	1.04
Thr	8	0.36 ± 0.061	0.36 ± 0.056	1.00	4	76.9 ± 7.70	75.0 ± 4.65	1.03
Trp	8	0.14 ± 0.048	0.11 ± 0.023	1.27	4	88.8 ± 3.66	83.9 ± 2.99	1.06
Val	8	0.54 ± 0.086	0.55 ± 0.092	0.98	4	80.6 ± 3.67	77.9 ± 4.27	1.03
Dispensable AA, %								
Ala	6	0.43 ± 0.056	0.42 ± 0.031	1.02	4	76.0 ± 9.90	70.9 ± 7.97	1.07
Asp	6	0.62 ± 0.087	0.61 ± 0.029	1.02	4	77.4 ± 7.22	72.9 ± 4.91	1.06
Cys	6	0.27 ± 0.051	0.23 ± 0.027	1.17	4	86.5 ± 3.23	80.4 ± 4.91	1.08
Glu	6	3.18 ± 0.812	2.35 ± 0.212	1.35	4	92.3 ± 2.48	86.7 ± 2.55	1.06
Gly	5	0.45 ± 0.033	0.34 ± 0.168	1.32	3	85.2 ± 18.58	71.5 ± 21.22	1.19
Pro	5	1.04 ± 0.269	1.01 ± 0.146	1.03	2	221.9 ± 150.12	95.0 ± 15.63	2.34
Ser	6	0.44 ± 0.117	0.37 ± 0.052	1.19	4	85.7 ± 2.71	80.6 ± 4.50	1.06
Tyr	6	0.32 ± 0.067	0.28 ± 0.058	1.14	4	84.6 ± 5.08	79.4 ± 4.20	1.07
Energy content (kcal/kg DM)								
GE	5	4,419 ± 130.8	4,439 ± 145.6	1.00				
DE	6	3,939 ± 109.9	3,699 ± 250.8	1.06				
ME	7	3,831 ± 107.6	3,577 ± 237.8	1.07				

[1] Mean values are sourced from articles that have direct comparisons of wheat and barley. Literature cited: Bolarinwa and Adeola, 2016; Cervantes-Pahm et al., 2014; Heyer et al., 2022; Koo et al., 2018; Lee et al., 2020; McGhee and Stein, 2018; McGhee and Stein, 2020; Park et al., 2016; Schemmer et al., 2020; Tan et al., 2021; Xie et al., al., 2017; Zhou et al., 2016

[2] Number of wheat/barley comparisons from collected publications.

Table 5.8 Nutrient and amino acid composition and standardized ileal digestibility (SID) of wheat compared to sorghum (as-fed basis)[1]

	Chemical composition (%, Mean ± SD)				SID (%, Mean ± SD)			
Item	N[2]	Wheat	Sorghum	Ratio	N[2]	Wheat	Sorghum	Ratio
Nutrient composition, %								
DM	8	89.5 ± 1.75	88.7 ± 1.61	1.01				
CP	8	11.8 ± 1.17	9.3 ± 1.12	1.27	3	84.9 ± 8.89	78.5 ± 11.46	1.08
EE	8	2.1 ± 0.59	2.9 ± 0.69	0.72				
Ash	7	1.6 ± 0.29	1.3 ± 0.40	1.23				
ADF	6	2.9 ± 0.54	2.9 ± 0.50	1.00				
NDF	6	13.2 ± 4.01	9.8 ± 3.70	1.35				
Ca	2	0.12 ± 0.099	0.03 ± 0.014	4.00				
P	2	0.37 ± 0.113	0.27 ± 0.007	1.37				
Starch	6	56.7 ± 5.78	62.4 ± 6.82	0.91				
Indispensable AA, %								
Arg	6	0.54 ± 0.051	0.32 ± 0.038	1.69	3	87.3 ± 0.76	83.0 ± 8.30	1.05
His	6	0.26 ± 0.032	0.20 ± 0.044	1.30	3	85.9 ± 1.71	75.4 ± 1.67	1.14
Ile	6	0.39 ± 0.032	0.34 ± 0.051	1.15	3	83.5 ± 4.75	78.3 ± 4.18	1.07
Leu	6	0.74 ± 0.082	1.09 ± 0.160	0.68	3	85.0 ± 3.82	81.7 ± 4.72	1.04
Lys	6	0.34 ± 0.022	0.22 ± 0.067	1.55	3	75.7 ± 3.29	71.8 ± 5.06	1.05
Met	6	0.17 ± 0.019	0.14 ± 0.015	1.21	3	86.6 ± 2.24	81.6 ± 4.03	1.06
Phe	6	0.51 ± 0.073	0.43 ± 0.054	1.19	3	87.2 ± 2.86	71.3 ± 16.21	1.22
Thr	6	0.32 ± 0.043	0.27 ± 0.029	1.19	3	75.7 ± 9.60	73.2 ± 4.99	1.03
Trp	6	0.14 ± 0.021	0.08 ± 0.020	1.75	3	83.2 ± 2.16	76.1 ± 2.43	1.09
Val	6	0.48 ± 0.036	0.44 ± 0.067	1.09	3	80.1 ± 5.35	77.7 ± 3.78	1.03
Dispensable AA, %								
Ala	6	0.40 ± 0.020	0.72 ± 0.082	0.56	3	73.4 ± 10.77	79.8 ± 7.03	0.92
Asp	6	0.59 ± 0.056	0.54 ± 0.052	1.09	3	75.6 ± 7.97	77.7 ± 5.60	0.97
Cys	6	0.25 ± 0.027	0.17 ± 0.031	1.47	3	86.8 ± 3.63	73.6 ± 4.61	1.18
Glu	6	3.08 ± 0.500	1.81 ± 0.430	1.70	3	93.5 ± 1.39	82.4 ± 4.69	1.13
Gly	6	0.45 ± 0.025	0.29 ± 0.054	1.55	3	82.4 ± 16.16	74.7 ± 22.6	1.10
Pro	6	1.09 ± 0.210	0.64 ± 0.142	1.70	2	133.5 ± 5.37	137.0 ± 9.26	0.97
Ser	5	0.46 ± 0.128	0.34 ± 0.024	1.35	3	86.8 ± 3.62	81.6 ± 2.73	1.06
Tyr	4	0.31 ± 0.059	0.27 ± 0.078	1.15	1	77.1	70.8	1.09
Energy content (kcal/kg DM)								
GE	5	4,341 ± 116.5	4,368 ± 145.9	0.99				
DE	6	3,913 ± 115.7	3,959 ± 152.8	0.99				
ME	6	3,790 ± 128.5	3,851 ± 178.3	0.98				

[1] Mean values are sourced from articles that have direct comparisons of wheat and sorghum. Literature cited: Abelilla and Stein, 2019; Bolarinwa and Adeola, 2016; Cervantes-Pahm et al., 2014; Jaworski et al., 2015; McGhee and Stein, 2020; Pan and An, 2020; Rodriguez et al., 2020; Xie et al., 2017.

[2] Number of wheat/sorghum comparisons from collected publications.

Table 5.9 Nutrient and amino acid composition of wheat co-products (as-fed basis)[1]

	Wheat bran		Wheat middlings		Wheat millrun		Wheat shorts		Wheat red dog	
Item	N[2]	Mean ± SD	N[2]	Mean ± SD	N[2]	Mean ± SD	N[2]	Mean ± SD	N[2]	Mean ± SD
Nutrient composition, %										
DM	24	89.2 ± 1.86	47	89.1 ± 1.41	4	89.2 ± 0.87	7	89.9 ± 2.96	7	90.8 ± 2.07
CP	26	16.0 ± 0.90	47	16.5 ± 1.77	4	16.6 ± 0.65	20	16.3 ± 2.04	19	13.8 ± 1.21
EE	19	3.1 ± 0.94	44	4.0 ± 1.12	4	3.9 ± 0.91	14	3.1 ± 0.72	14	2.4 ± 0.61
Ash	20	5.2 ± 0.70	37	5.2 ± 0.82	4	5.5 ± 0.28	14	3.3 ± 1.08	14	2.7 ± 1.79
CF	5	10.2 ± 1.23	16	8.4 ± 0.67	4	8.9 ± 1.36	17	5.3 ± 0.94	16	2.6 ± 1.04
ADF	20	11.3 ± 1.63	33	10.5 ± 1.51	5	13.6 ± 2.10	21	6.3 ± 2.78	15	2.5 ± 1.05
NDF	21	39.4 ± 4.01	33	34.7 ± 4.20	5	34.4 ± 3.43	21	25.0 ± 5.99	15	11.6 ± 4.04
TDF	12	42.9 ± 4.35	17	37.8 ± 4.24	1	38.0	1	19.3	1	13.9
IDF	12	39.6 ± 4.46	16	34.7 ± 4.72	1	35.5	1	15.2	1	12.8
SDF	12	3.2 ± 0.83	16	2.9 ± 1.28	1	2.4	1	4.1	1	1.1
Ca	7	0.11 ± 0.034	35	0.14 ± 0.127	3	0.14 ± 0.051	16	0.05 ± 0.029	18	0.16 ± 0.252
P	8	0.99 ± 0.135	35	1.05 ± 0.169	3	0.97 ± 0.136	16	0.40 ± 0.279	18	0.30 ± 0.092
Starch	11	14.5 ± 3.29	30	20.3 ± 3.21	2	20.3 ± 7.19	11	33.3 ± 7.94	13	54.9 ± 8.89
Indispensable AA, %										
Arg	8	1.03 ± 0.139	29	1.07 ± 0.073	1	1.09	8	0.93 ± 0.149	7	0.68 ± 0.131
His	8	0.45 ± 0.074	29	0.43 ± 0.028	1	0.42	8	0.39 ± 0.041	7	0.32 ± 0.030
Ile	8	0.49 ± 0.039	29	0.52 ± 0.038	1	0.52	8	0.49 ± 0.037	7	0.44 ± 0.063
Leu	8	0.94 ± 0.096	29	1.01 ± 0.070	1	1.01	8	0.99 ± 0.048	7	0.91 ± 0.075
Lys	8	0.62 ± 0.070	29	0.70 ± 0.049	1	0.74	8	0.59 ± 0.078	7	0.45 ± 0.110
Met	8	0.23 ± 0.021	29	0.24 ± 0.020	1	0.23	8	0.25 ± 0.028	7	0.24 ± 0.059
Phe	8	0.60 ± 0.095	29	0.64 ± 0.048	1	0.65	8	0.65 ± 0.071	7	0.62 ± 0.076
Thr	8	0.49 ± 0.053	29	0.52 ± 0.034	1	0.52	8	0.51 ± 0.062	7	0.41 ± 0.072
Trp	5	0.18 ± 0.049	29	0.17 ± 0.030	1	0.19	6	0.20 ± 0.031	7	0.16 ± 0.015
Val	8	0.72 ± 0.071	29	0.76 ± 0.056	1	0.75	8	0.73 ± 0.084	7	0.60 ± 0.064
Dispensable AA, %										

Ala	5	0.79 ± 0.071	27	0.77 ± 0.052	1	0.79	2	0.85 ± 0.088	1	0.63
Asp	5	1.12 ± 0.052	27	1.13 ± 0.084	1	1.15	2	1.24 ± 0.186	1	0.98
Cys	5	0.36 ± 0.051	27	0.32 ± 0.016	1	0.30	2	0.30 ± 0.193	1	0.29
Glu	5	2.85 ± 0.296	27	2.96 ± 0.191	1	2.72	2	2.84 ± 0.327	1	3.58
Gly	5	0.85 ± 0.029	27	0.84 ± 0.041	1	0.85	2	0.84 ± 0.023	1	0.69
Pro	3	0.92 ± 0.058	17	0.95 ± 0.106	1	0.93	1	0.92	1	1.13
Ser	5	0.61 ± 0.049	27	0.59 ± 0.065	1	0.59	2	0.69 ± 0.095	1	0.58
Tyr	5	0.37 ± 0.073	25	0.40 ± 0.051	1	0.46	2	0.36 ± 0.134	1	0.43

[1] Literature cited: Abelilla and Stein, 2019; Acosta et al., 2017; Adedokun and Adeola, 2020; Adedokun et al., 2019; Berrocoso et al., 2015; Casas et al., 2018; Chee et al., 2005; De Jong et al., 2014; Eklund et al., 2014; Espinosa et al., 2024; Franceschina et al., 2015; He et al., 2021; Huang et al., 2013; Huang et al., 2014; Huang et al., 2015; Jaworski et al., 2015; Jaworski et al., 2016; Jaworski et al., 2017; Jha et al., 2012; Kim et al., 2022; Kraler et al., 2014; Lee et al., 2020; Lee et al., 2022; Lowell et al., 2015; Lyu et al., 2023; Navarro et al., 2018; Nortey et al., 2008; Oliviera et al., 2020; Oliviera et al., 2022; Poulsen and Blaabjerg, 2016; Salyer et al., 2012; She et al., 2015; Son et al., 2023; Stewart et al., 2013; Trindade Neto et al., 2020; Wesendonck et al., 2013; Wu et al., 2016; Zhang et al., 2020; Zhao et al., 2018; Zhao et al., 2018; Zhao et al., 2020.

[2] The number of wheat co-product samples from collected publications.

Table 5.10 Standardized ileal digestibility (SID) of CP and AA in wheat co-products[1]

	Wheat bran		Wheat middlings[2]		Wheat middlings[3]		Wheat shorts		Wheat red dog	
Item	N[4]	Mean ± SD	N[4]	Mean ± SD	N[4]	Mean ± SD	N[4]	Mean ± SD	N[4]	Mean ± SD
SID of CP	3	67.5 ± 5.83	11	70.6 ± 6.07	10	61.5	7	85.9 ± 10.52	6	88.1 ± 4.78
SID of Indispensable AA, %										
Arg	5	83.0 ± 6.55	17	83.3 ± 6.06	10	81.4	7	91.8 ± 5.30	6	92.1 ± 3.01
His	5	76.8 ± 9.59	17	80.0 ± 2.61	10	77.7	5	93.9 ± 1.63	6	89.8 ± 7.34
Ile	5	73.9 ± 5.11	17	74.9 ± 4.39	10	69.4	7	86.1 ± 11.05	6	89.7 ± 3.20
Leu	5	76.3 ± 5.38	17	78.5 ± 4.12	10	72.5	7	90.9 ± 5.17	6	91.7 ± 3.76
Lys	5	69.3 ± 9.61	17	70.2 ± 7.55	10	45.9	7	87.9 ± 4.20	6	83.1 ± 6.14
Met	5	76.9 ± 7.83	17	79.8 ± 4.51	10	73.6	7	91.3 ± 3.03	6	93.0 ± 1.57
Phe	5	75.9 ± 6.99	17	79.3 ± 3.63	10	69.8	5	94.2 ± 1.66	6	92.4 ± 3.51
Thr	5	68.1 ± 9.35	17	70.6 ± 4.53	10	62.7	7	86.7 ± 5.53	6	86.5 ± 2.24
Trp	2	66.1 ± 18.24	17	77.6 ± 7.27	10	70.5	5	89.0 ± 4.07	6	89.2 ± 1.28
Val	5	73.7 ± 6.00	17	75.2 ± 5.70	10	63.7	7	90.1 ± 4.62	6	86.8 ± 7.42
SID of Dispensable AA, %										
Ala	4	69.0 ± 6.30	15	68.2 ± 6.16	10	54.8	0	---	1	77.5
Asp	4	68.9 ± 3.32	15	72.1 ± 4.47	10	66.4	0	---	1	77.1
Cys	3	73.3 ± 1.86	15	75.6 ± 3.75	10	73.7	2	82.5 ± 0.71	1	57.4
Glu	4	82.7 ± 3.68	15	86.0 ± 2.84	10	81.8	0	---	1	91.9
Gly	3	71.9 ± 6.01	15	66.5 ± 8.30	0	---	0	---	0	---
Pro	3	85.8 ± 13.49	2	120.5 ± 9.12	0	---	0	---	0	---
Ser	4	77.4 ± 3.50	15	76.2 ± 4.83	10	72.5	0	---	1	74.9
Tyr	4	75.8 ± 4.43	13	76.0 ± 4.94	10	68.4	0	---	1	85.5

[1] Adedokun et al., 2019; Casas and Stein, 2018; Eklund et al., 2014; Espinosa et al., 2024; Huang et al., 2012; Huang et al., 2014; Jaworski et al., 2016; Koo et al., 2017; Lee et al., 2020; Lee et al., 2021; Libao-Mercado et al., 2006; Lyu et al., 2020; Lyu et al., 2023; Neto et al., 2020; Oliviera et al., 2020; Woyengo and Zijlstra, 2021; Zhao et al., 2018. There were no journal articles with SID values for wheat millrun.

[2] Non-heat damaged wheat middlings samples.

[3] Heat damaged wheat middlings samples from Casa and Stein, 2018.

[4] The number of wheat co-product samples from collected publications.

Table 5.11 Energy content of wheat co-products[1]

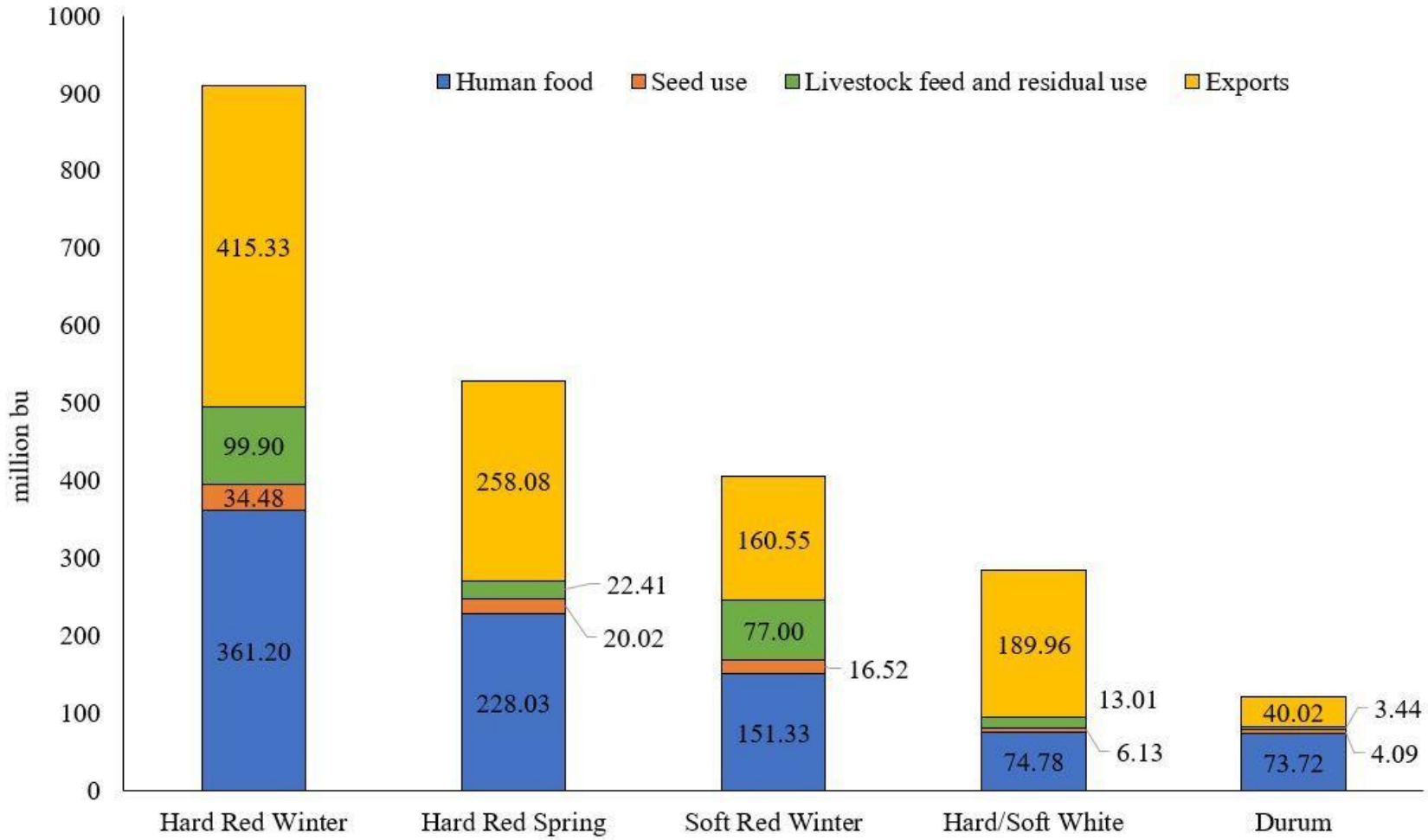
	Wheat bran		Wheat middlings		Wheat shorts		Wheat red dog	
Item	N[2]	Mean ± SD	N[2]	Mean ± SD	N[2]	Mean ± SD	N[2]	Mean ± SD
Energy content (kcal/kg DM)								
GE	11	4,551 ± 67.5	20	4,468 ± 67.3	9	4,426 ± 286.7	10	4,343 ± 175.0
DE	12	2,796 ± 236.9	21	2,982 ± 141.2	9	3,344 ± 234.7	10	3,665 ± 238.1
ME	12	2,636 ± 219.3	20	2,872 ± 145.1	9	3,152 ± 185.6	10	3,473 ± 170.9
Calculated NE[3]	10	1,950 ± 179.7	11	2,085 ± 111.7	9	2,343 ± 163.8	10	2,578 ± 170.9

[1] Adedokun et al., 2019; Casas et al., 2018; Espinosa et al., 2024; Huang et al., 2012; Huang et al., 2014; Jaworski et al., 2016; Kim et al., 2022; Lyu et al., 2020; Lyu et al., 2023; Stewart et al., 2013; Woyengo and Zijlstra, 2021.

[2] The number of wheat co-product samples from collected publications.

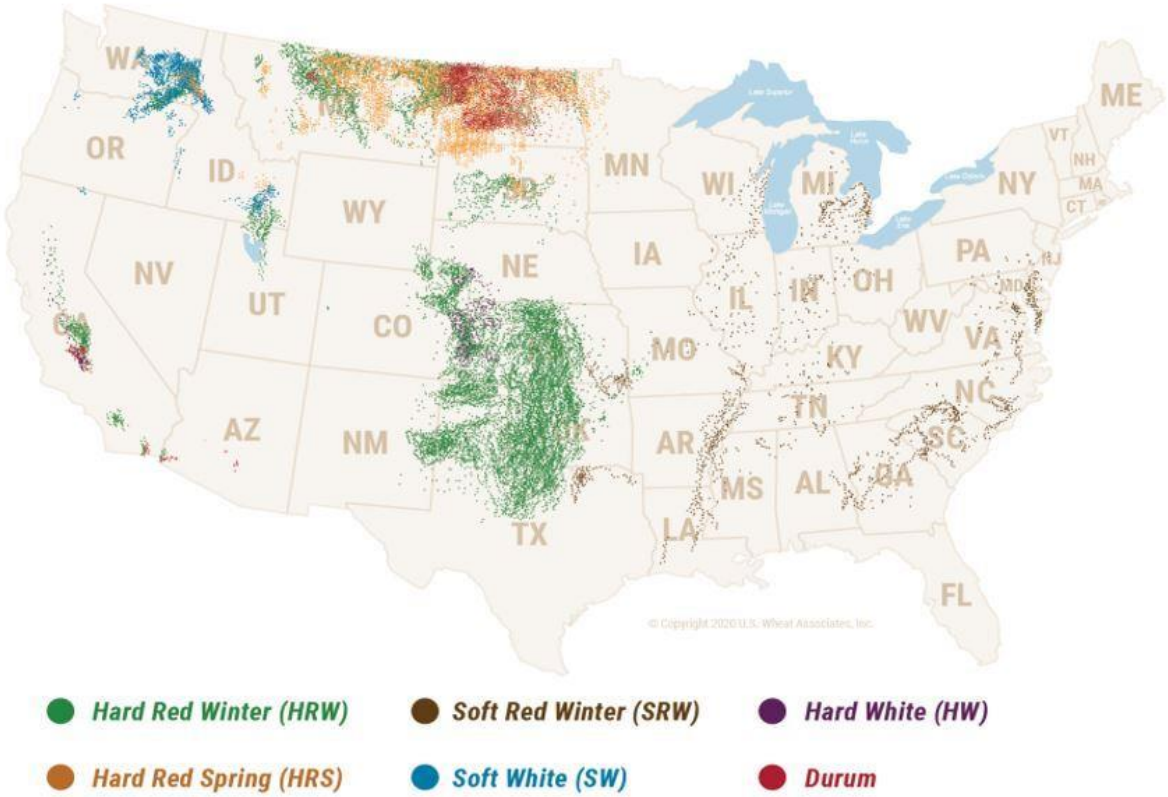
[3] Calculated using Eq. 1-8 from NRC (2012). Net energy (kcal/kg DM) = (0.700 × DE) + (1.61 × EE) + (0.48 × Starch) – (0.91 × CP) – (0.87 × ADF).

Figure 5.1 United States wheat class production by end use[1]



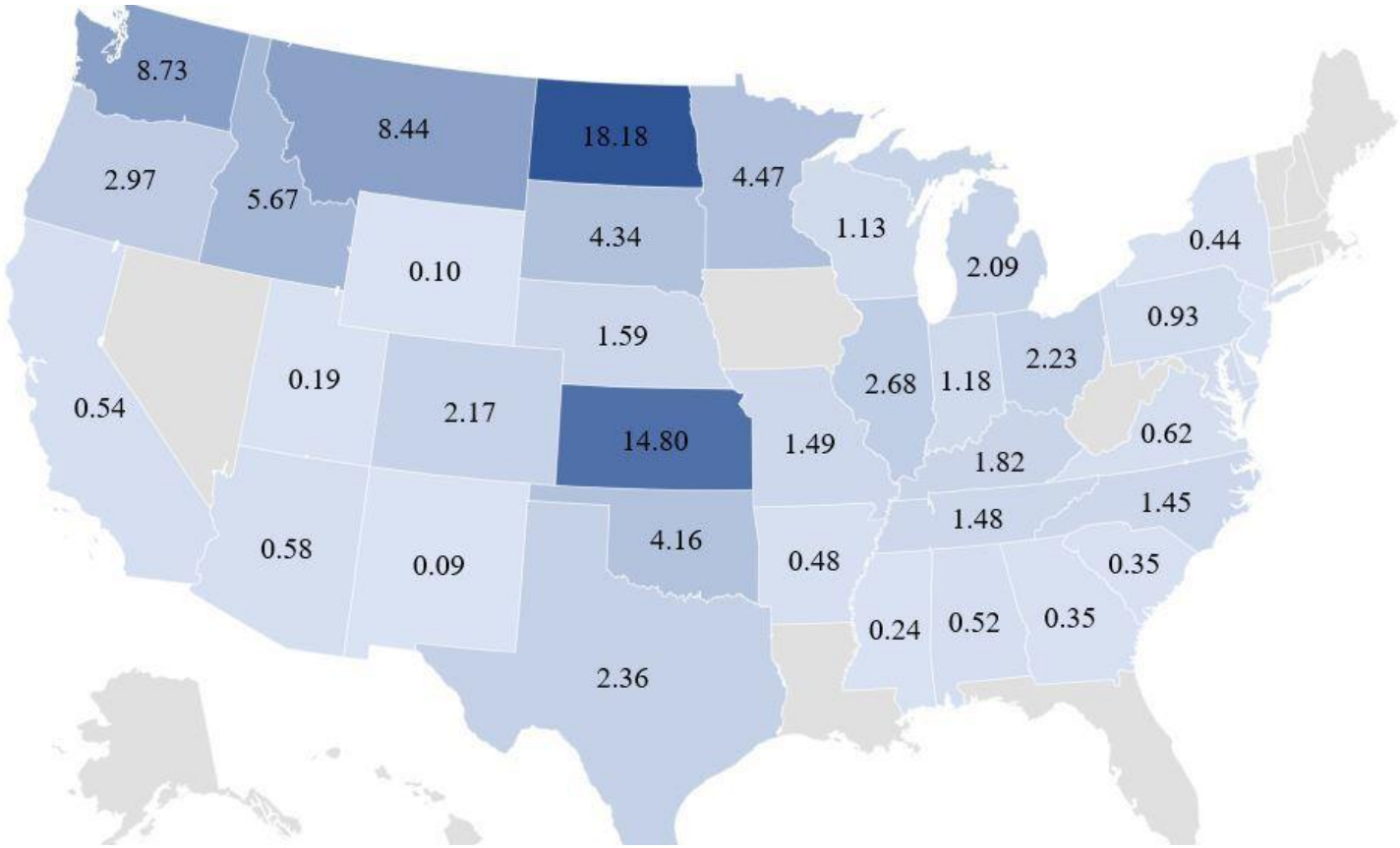
[1]USDA, 2023.

Figure 5.2 United States wheat class by state[1]



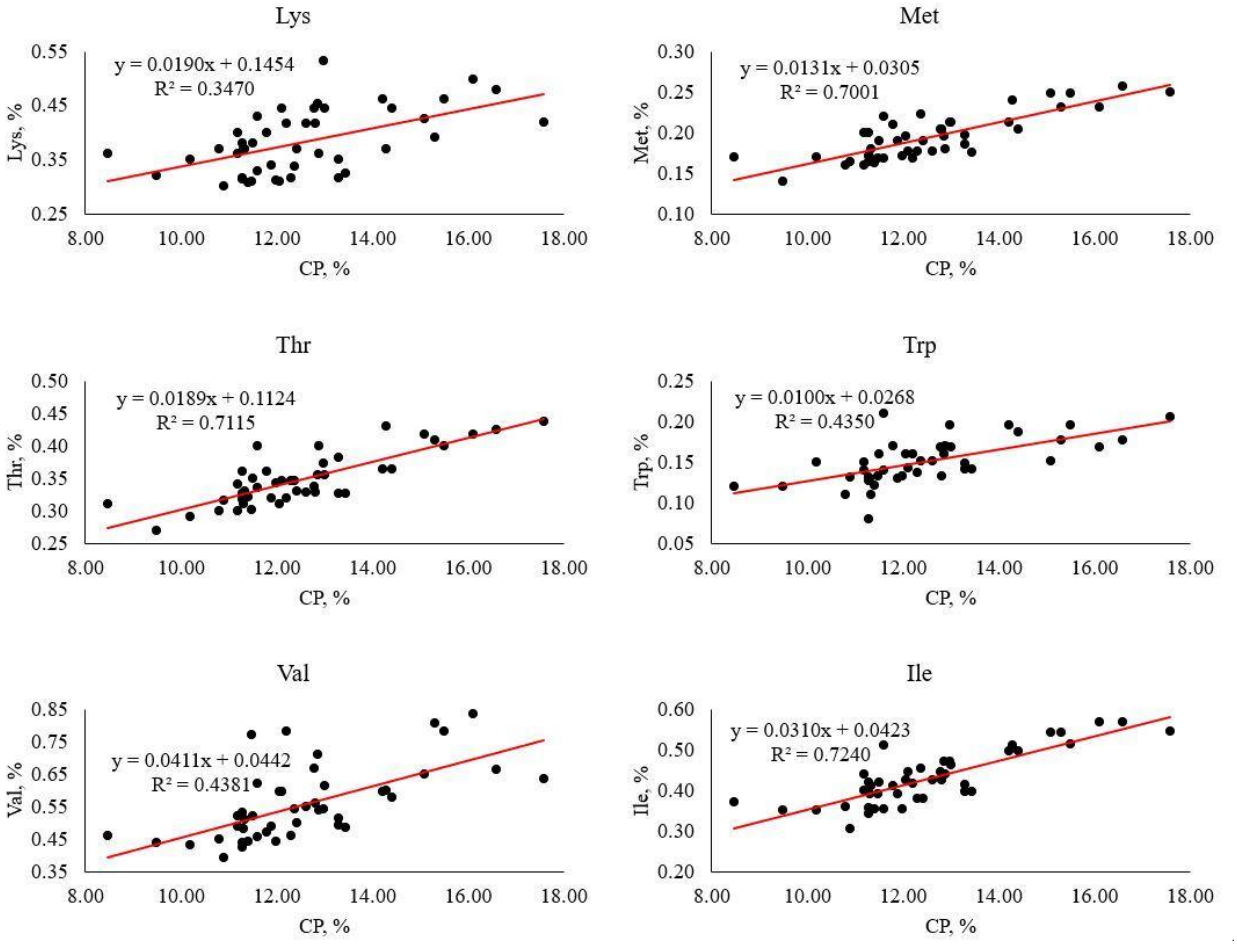
[1] U.S. Wheat Associates, 2022. The map is a representation of wheat class production density by geographical location.

Figure 5.3 Percentage of United States wheat production by state[1]



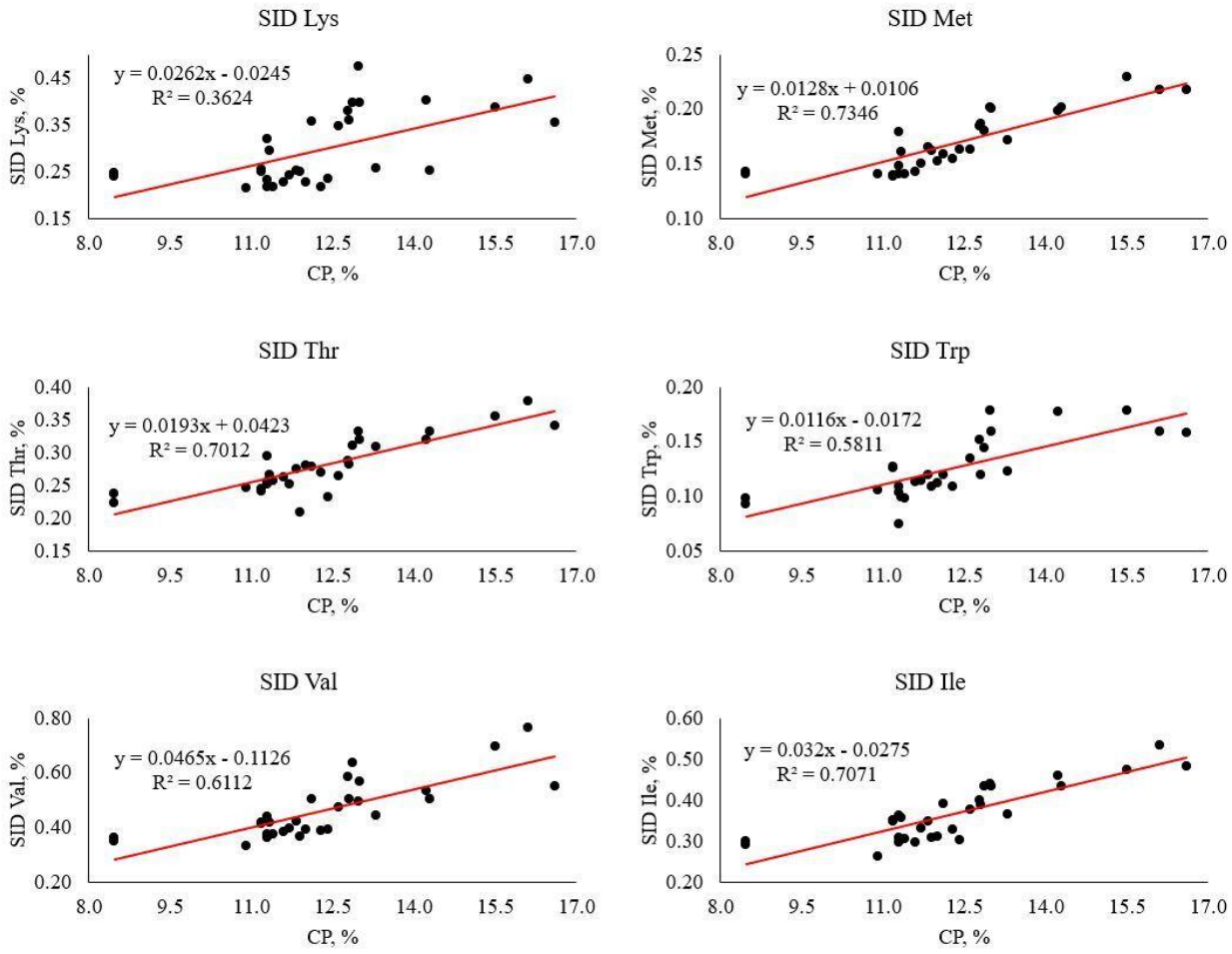
[1]USDA, 2023.

Figure 5.4 Simple linear regression to predict total Lys, Met, Thr, Trp, Val, and Ile concentration using the crude protein content in wheat[1]



[1] Literature Cited: Abelilla and Stein, 2019; Bloxham et al., 2018; Bolarinwa et al., 2018; Cervantes-Pahm et al., 2014; De Jong et al., 2014; Eklund et al., 2014; Fan et al., 2017; Heyer et al., 2022; Jaworski et al., 2015; Jeon et al., 2019; Koo et al., 2018; Lee et al., 2020; Le et al., 2016; McGhee and Stein, 2018; McGhee and Stein, 2020; Oliveria et al., 2020; Pan and An, 2020; Park et al., 2016; Rodriguez et al., 2020; Rosenfelder et al., 2015; Schemmer et al., 2020; She et al., 2015; Tan et al., 2021; Wang et al., 2018; Xie et al., 2017; Zhang et al., 2020; Zhao et al., 2019; Zhou et al., 2016. There were 45 samples for all AA except Trp which had 43 samples.

Figure 5.5 Simple linear regression to predict content of standardized ileal digestible (SID) Lys, Met, Thr, Trp, Val, and Ile in wheat in relation to the crude protein content[1]



[1] Literature Cited: Cervantes-Pahm et al., 2014; Lee et al., 2020; McGhee and Stein, 2018; Oliveria et al., 2020; Rodriguez et al., 2020; Rosenfelder et al., 2015; Tan et al., 2021; Wang et al., 2018; Zhao et al., 2019. There were 30 samples for all AA except Trp which had 28 samples.

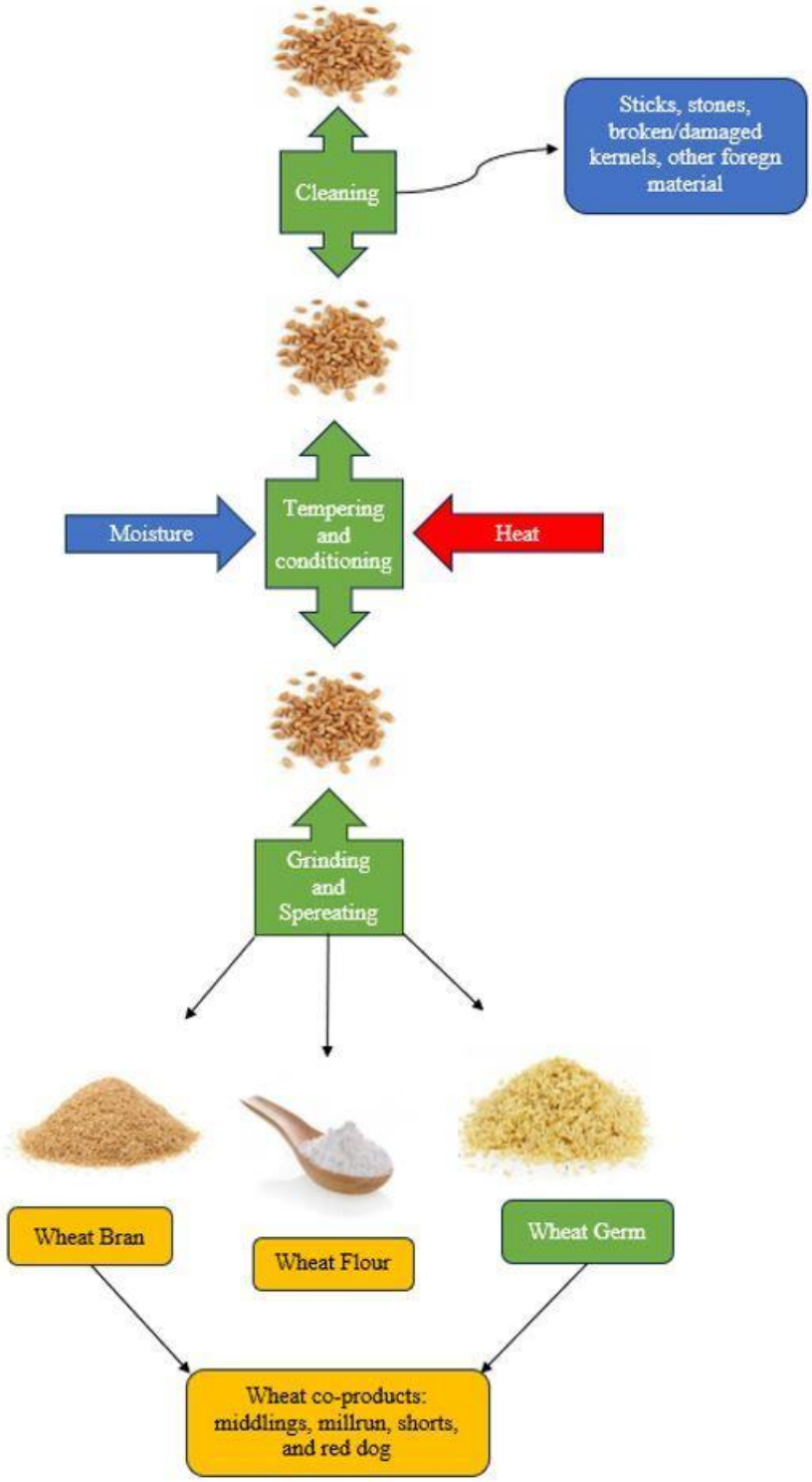



Figure 5.6 Wheat milling flow diagram